



Quantitation with QuantAnalysis Tutorial

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Preliminary remarks

Intention of this document

This document shortly describes how to perform a quantitation with the QuantAnalysis software (version 1.5). Quantitation consists of acquiring data files from calibration solutions, quality control samples (QCs), and unknown samples. The acquisition of those data files is normally done within a sequence, which is set up in the Agilent ChemStation program, but also a series of individually acquired data files can be used (an example for each case is given). Quantitative data analysis is performed after the data acquisition ("post-acquisition") within QuantAnalysis.

Two examples (using data files available on the tutorial CD-ROM) are given to illustrate the task flow of quantitation with QuantAnalysis and some of its features:

1) Quantitation with a Agilent ChemStation sequence file:

Single component quantitation with internal standard calibration.

Target compound: alprazolam d0 („d0“), a chlorinated benzodiazepine (MW 308)

Internal standard (ISTD): penta-deuterated derivative, alprazolam d5 („d5“, MW 313)

The sequence consists of four standard solutions containing the target compound d0 at concentrations of 2, 10, 20 and 100 pg/μl, and the ISTD (d5) at a constant concentration of 10 pg/μl. Two solutions, which contain 5 or 50 pg/μl d0, and 10 pg/μl d5, function as „unknown“ samples. Furthermore three injections of a blank solution were used.

2) Quantitation of a set of data files obtained individually:

Multi-component quantitation with external calibration.

Target compounds: three carbamates:

Propamocarb („PCB“), MW 188

Carbendazim („CBZ“), MW 191

Pirimicarb („PRC“), MW 238

The series consists of eight individually acquired data files. Four standard solutions containing each compound at a concentration level of 0.1, 1, 10 and 100 pg/μl were used with an injection volume of 10 μl. Three additional runs using only an injection volume of 5 μl were used to get supplementary calibration points (simulating concentration levels of 0.5, 5 and 50 pg/μl). The solution containing 100 pg/μl of each carbamate furthermore functioned as „unknown“ sample (injection volume 3 μl).

Note *Detailed explanations about working with QuantAnalysis are available from the online help file within the program!*

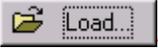
Explanation of file types important for the task flow within QuantAnalysis

Note *It is important to know and understand the different file types, which are to be handled from QuantAnalysis:*

***.btc:** *Batch-file, created from QuantAnalysis, It contains the content of the worktable (see ch. 1.2.1).*

An already existing batch file can be opened in QuantAnalysis via the -button or by File | Open....

seqtable.csv: *Sequence file, created from Agilent ChemStation during a sequence run. It contains those informations, which were entered within the sequence table of Agilent ChemStation. (see ch. 2.1 and manual of Agilent ChemStation for further explanations).*

You can not open a csv-file with the -button or by File | Open.... These commands only work for batch-files (.btc)! The file seqtable.csv can only be loaded into the worktable by using the -Button (see fig. 2)*

***.d:** *Name of an single analysis file (one single chromatographic LC/MS run) and of the folder, in which the data of this run are stored.*

An analysis file can not directly been opened or loaded via any of the commands mentioned above. If the file names belonging to a quantitation series can not be read in from an sequence file, they have to be entered manually into the worktable (see ch. 2.2.2).

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1 Start QuantAnalysis and General Overview

1.1 Start QuantAnalysis

The program is accessible from windows-explorer within the program directory:
c:\program files \ Bruker Daltonics \ QuantAnalysis \  QuantAnalysis.exe
or via the start button in the task bar:

Start | Programs | Bruker Daltonics |  QuantAnalysis.

You can also create a link on your desktop by „dragging and dropping“ QuantAnalysis.exe from the windows-explorer to it.

Click **Log On** to close the dialog box, a password is not defined. If several users are installed, log in with the correct username and password.

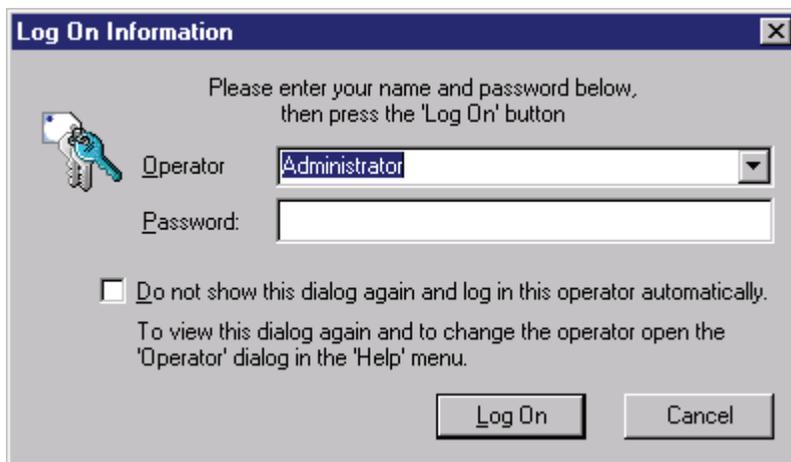


Figure 1 Login Information Dialog

1.2 General Overview: Display modes

The program has three different display windows („Work Table“, „Chromatogram/Spectrum“ and „Calibration“, description see below), which can be displayed individually or all together („Nested View“). To toggle between the display modes click on the corresponding tags at the bottom of the screen (red ellipse, **fig. 1**). On ending QuantAnalysis the actual display mode remains active and shows up on the next program start.

Note

The „Nested view“ can be adjusted by dragging the border lines between the windows (blue arrows, fig. 1). After the first start of the program it may happen that the „Nested view“ seems to show only one of the three windows. In this case please rearrange the border lines to get an appropriate view.

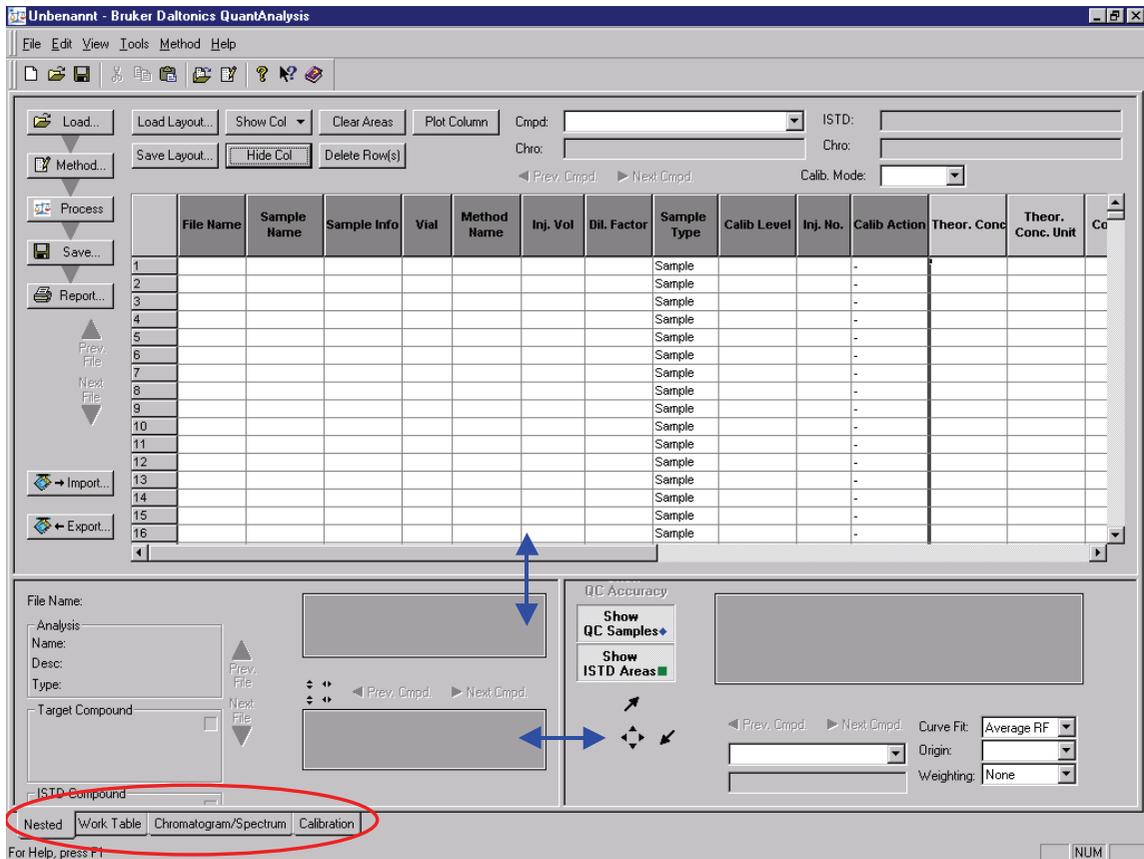


Figure 2 „Nested view“ of QuantAnalysis

1.2.1 Work Table

The work table contains all information that is associated with the quantitation of a batch of samples. By defining and saving this table you create the so-called **batch-file** (file extension *.btc). This batch file allows for easy access to the complete series of files if you later want to review the results or to reprocess them.

In future versions of QuantAnalysis all changes which are made to a primary calibration and quantitation will be saved and will be **back-trackable**, so that different statuses can be visualized.

The work table has **two major areas**:

a) „Sample-specific fields“: for example FileName, SampleInfo, VialPosition, InjectionVolume, SampleType, CalibrationLevel,... This part shows dark gray headers.

b) „Compound- and sample-specific fields“: for example TheoreticalConcentration, RetentionTime, ConcentrationInternalStandard... This part shows light gray headers. In the case of a **multi-component calibration** each of these fields exists multiple times, one time for each compound. Additionally to the informations which are needed before performing the quantitation the results of the quantitation will be found here (fields like AreaTargetCompound, AreaSTDCCompound, RetentionTime, CalculatedConcentration...).

The last column („**Accuracy**“) will be calculated only for two types of samples (Calibration and QualityControl, QC). It is calculated as the ratio between the calculated concentration (evaluation of the peak area against the current calibration curve) and the corresponding (known) theoretical value and therefore easily allows for an assessment of the quality of the integration results and calibration curve.

The similarity of the work table to the sequence table under the Agilent ChemStation software is intended. On one hand, you actually can import a sequence that already was run under Agilent ChemStation. On the other hand you will also be able in future versions of the software to do the experimental setup under QuantAnalysis, to export the necessary informations to Agilent ChemStation and immediately start the sequence since the information that is needed in Agilent ChemStation is a kind of a subset of the work table.

1.2.2 Chromatogram/Spectrum Display

After you have done a quantitation the individual compound-peaks and averaged MS-spectra for each peak will be displayed in the Chromatogram/Spectrum display. You can browse through the results for all files and compounds. If compound peaks are not recognized during the quantitation process or not properly integrated, the integration can be corrected by means of a manual integration mode. In this mode each compound peak can easily be reintegrated individually without need of reprocessing the whole batch of files.

1.2.3 Calibration Display

After the quantitation, the calibration display shows the calibration curve. The type of calibration curve and the actually displayed compound can be chosen from drop-down fields.

Note

The contents of the different windows are dynamically linked. Changes which are made in one of the windows are instantaneously updated in the others. For example: if you choose another kind of calibration curve in the calibration display all values in the worktable will be recalculated and displayed immediately. This allows for a very quick and easy adjusting of parameters or (if necessary) for corrections like manual integration of single peaks. In this case the corresponding calibration point will automatically be adjusted and replaced at once.

2 Task flow of quantitation with QuantAnalysis

2.1 Example 1: Quantitation with a Agilent ChemStation sequence file

2.1.1 Remarks

This example shows a **single component quantitation** with **internal standard calibration**:

Target compound: alprazolam d0 („d0“), a chlorinated benzodiazepine (MW 308)
Internal standard (ISTD): penta-deuterated derivative, alprazolam d5 („d5“, MW 313)

The data files were **acquired by using a sequence** in the Agilent ChemStation software. The sequence consists of four standard solutions containing the target compound d0 at concentrations of 2, 10, 20 and 100 pg/μl, and the ISTD (d5) at a constant concentration of 10 pg/μl. Two solutions, which contain 5 or 50 pg/μl d0, and 10 pg/μl d5, function as „unknown“ samples. Furthermore three injections of a blank solution were used.

2.1.2 Step 1: Generation of the batch file

- Switch to the full-screen display for the work table (red ellipse in **fig. 1**).
- **Import the sequence** file: click on the  Import...-Button (red ellipse in **fig. 2**), change to the directory where the sequence was saved during the installation process of this tutorial, choose „**seqtable.csv**“ and click on **Open** . The work table will display the names of the chromatogram files and all other informations which were saved with the sequence (**fig. 2**) like sample names, vial position, injection volumes...

Note *You can not open a csv-file with the  Load...-button or by File | Open.... These commands only work for batch-files (*.btc)!*

→ **Adjust the work table layout:** Select those columns which you need and deactivate all other columns. To do so mark a column by clicking on its header and click on the button **Hide column**. To redisplay a column click on **Show column** and select it in the drop-down field. Make sure that the columns „**SampleType**“ and „**Calibration Level**“ remain displayed. The width of a column can also be optimized if for example an entry is not shown in full length or if a column is much wider than necessary: move the cursor to a separation line between two fields of column headers and drag the line (left mouse button kept pressed; green arrow in **fig. 2**) to a more appropriate position.

Note

*Adjust the layout of the work table to the kind of quantitation you are going to do. For example if you want to do an external calibration hide all columns which define parameters of an internal standard. Save an optimized layout with **Save Layout** (blue ellipse in **fig. 2**; file extension *.qal) or reload a formerly defined layout with **Load Layout**.*

The screenshot shows the Bruker Daltonics QuantAnalysis software interface. The window title is "Seqtable.btc - Bruker Daltonics QuantAnalysis". The menu bar includes File, Edit, View, Tools, Method, and Help. The toolbar contains icons for Load, Save, Print, and other functions. The main area is a table with the following columns: File Name, Sample Name, Sample Info, Vial, Method Name, Inj. Vol, Dil. Factor, Sample Type, Calib Level, Inj. No., Calib Action, Theor. Conc., Theor. Conc. Unit, Conc. ISTD, Conc. ISTD Unit, and RT Target Cmpd. The table contains 29 rows of data. The "Load Layout..." button in the toolbar is circled in blue, and the "Import..." button in the left sidebar is circled in red.

	File Name	Sample Name	Sample Info	Vial	Method Name	Inj. Vol	Dil. Factor	Sample Type	Calib Level	Inj. No.	Calib Action	Theor. Conc.	Theor. Conc. Unit	Conc. ISTD	Conc. ISTD Unit	RT Target Cmpd
1	etp00002.D	Blank	Blank	1	WASH.M	10	1	Sample	0	1	-					
2	etp00003.D	C20	Alp 20pg/100pg (d0/d5)	2	ALPQMS.M	10	0.05	Calib	1	1	Replace					
3	etp00004.D	C100	Alp 100pg/100pg (d0/d5)	3	ALPQMS.M	10	0.01	Calib	2	1	Replace					
4	etp00005.D	C200	Alp 200pg/100pg (d0/d5)	4	ALPQMS.M	10	0.005	Calib	3	1	Replace					
5	etp00006.D	C1000	Alp 1000pg/100pg (d0/d5)	5	ALPQMS.M	10	0.001	Calib	4	1	Replace					
6	etp00007.D	Blank	Blank	1	WASH.M	10	1	Sample	4	1	-					
7	etp00008.D	S50	Alp 50pg/100pg (d0/d5)	6	ALPQMS.M	10	1	Sample	4	1	-					
8	etp00009.D	S500	Alp 500pg/100pg (d0/d5)	7	ALPQMS.M	10	1	Sample	4	1	-					
9	etp00010.D	Blank	Blank	1	WASH.M	10	1	Sample	4	1	-					
10								Sample			-					
11								Sample			-					
12								Sample			-					
13								Sample			-					
14								Sample			-					
15								Sample			-					
16								Sample			-					
17								Sample			-					
18								Sample			-					
19								Sample			-					
20								Sample			-					
21								Sample			-					
22								Sample			-					
23								Sample			-					
24								Sample			-					
25								Sample			-					
26								Sample			-					
27								Sample			-					
28								Sample			-					
29								Sample			-					

Figure 3 Import of a Agilent ChemStation sequence file

→ **Load** the predefined layout “Ext_Std.qal” with **Load Layout** (the file can be found in the same directory as the seqtable.csv for this example).

→ **Enter necessary informations:**

QuantAnalysis of course needs some additional informations. To perform a quantitation you **must** define the entries in the columns „**Sample Type**“ and „**Calibration Level**“. The sample type (sample, calib, QC or blank; QC = quality control; sample of known concentration) has to be chosen from a drop-down menu. The calibration level can be given in arbitrary numbers (e.g. 1, 2, 3,...) or as text (e.g. low, medium, high... for quality control samples). Of course, for samples of the kind „sample“ and „blank“ no definition of „calibration level“ is needed.

Note *The calibration level is meant as a type of “name” or identification label for a certain concentration, but it is not the value of the concentration.*

→ **Change the sample type for the three blank runs into „blank“.**

In the current software version **concentration values**, which belong to the different calibration levels have to be defined later during the setup of the method (see step 2). You can enter the theoretical concentrations into the corresponding column in the work table, but the program does not yet read these values! Nevertheless it is helpful to enter the concentrations in the table, too. If the column „Theor.Conc.“ is left blank, the program itself enters the corresponding values during the batch-processing of the files. Some more columns **must** contain informations, because the program uses them during the batch-processing for a **validation of the worktable** before it starts to load the individual files. During this validation the following things are checked:

- **End of the worktable:** the first row that contains no entry in the field „**FileName**“ is considered to be the end of the batch. The worktable therefore may not contain rows which are left blank!
- A „**MethodName**“ must be given for every chromatogram and within a batch of files this method name must be the **same for all**.

→ In example 1 change „WASH.M“ into „ALPQMS.M“ (see **fig. 3**).

Note *The concept of method handling within QuantAnalysis differs significantly from method handling in Agilent ChemStation. Whereas in Agilent ChemStation it is possible to use different methods within the same sequence (for example to acquire chromatograms with different LC- or MS-parameter settings), it is not possible to handle data files within QuantAnalysis with different methods.*

- An injection volume (column „**Inj.Vol.**“) must be given which is different from zero. Injection volumes which differ from a default value (defined later during the setup of the method) are used to adjust the calculated results (compare example 2: calibration points for different concentration levels can be obtained from the same solution by using different injection volumes).
- The dilution factor (column „**Dil.Factor**“) must be different from zero (for example just use „1“ for all samples). Dilution factor and injection factor are both taken into consideration for the calculation of results according to:

$$C_{theor} = \frac{C_{Std}}{DilFact} * \frac{InjVol}{DefInjVol}$$

→ In example 1 change all values to „1“ where necessary.

- The injection number (column „**Inj.Number**“) must be different from zero. (For handling of multiple injections see chapter 3.1)
- For calibration- and quality control samples a „**Calibration level**“ must be defined. The program uses this level to read the corresponding concentration from the table defined in the method (see step 2).
 - Delete the entries for blanks and samples.
- For every calibration file a „**Calibration action**“ (new or replace) must be chosen.

	File Name	Sample Name	Sample Info	Vial	Method Name	Inj. Vol	Dil. Factor	Sample Type	Calib Level	Inj. No.	Calib Action	Theor. Conc.	Theor. Conc. Unit	Conc. ISTD	Con
1	alp00002.D	Blank	Blank	1	ALPGMS.M	10	1	Blank		1	-				
2	alp00003.D	C20	Alp 20pg/100pg (d0/d5)	2	ALPGMS.M	10	1	Calib	1	1	New				
3	alp00004.D	C100	Alp 100pg/100pg (d0/d5)	3	ALPGMS.M	10	1	Calib	2	1	New				
4	alp00005.D	C200	Alp 200pg/100pg (d0/d5)	4	ALPGMS.M	10	1	Calib	3	1	New				
5	alp00006.D	C1000	Alp 1000pg/100pg (d0/d5)	5	ALPGMS.M	10	1	Calib	4	1	New				
6	alp00007.D	Blank	Blank	1	ALPGMS.M	10	1	Blank		1	-				
7	alp00008.D	S50	Alp 50pg/100pg (d0/d5)	6	ALPGMS.M	10	1	Sampl		1	-				
8	alp00009.D	S500	Alp 500pg/100pg (d0/d5)	7	ALPGMS.M	10	1	Sampl		1	-				
9	alp00010.D	Blank	Blank	1	ALPGMS.M	10	1	Blank		1	-				
10								Sampl			-				
11								Sampl			-				

Figure 4 Corrected work table for example 1

→ Change the entries to „new“ („new“: an additional calibration point is added to the curve; „replace“: can be used for multiple injection of a sample; an already existing calibration point of this calibration level is deleted and replaced).

The work table for example 1 should now look like the one given in **fig. 3**.

- **Review the work table:** If you have entered all informations which are necessary or which you want to see there additionally, **please have a careful look over all entries**. After a batch was processed, some of the columns may not be edited anymore. If you try to make changes to these fields after batch processing the entries will be ignored. The following table shows which column in the actual software version can be edited before and/or after processing of the batch:

	before processing	after processing
File Name	X	
Sample Name	X	
Sample Info	X	
Vial	X	
Method Name	X	
Inj. Vol	X	
Dil. Factor	X	X
Sample Type	X	
Calib Level	X	X
Inj. Number	X	X
Calib Action	X	X
Acq. Date		
Operator		
Theor. Conc.		X
Theor. Conc. Unit		X
Conc. ISTD		X
Conc. ISTD Unit		X
RT Target Cmpd		
Area Target Cmpd		
Area ISTD Cmpd		
Ratio Area Target/ISTD		
Calc. Conc.		
Calc. Conc. Unit		X
Recovery		

Note *If you later after processing notice a mistake in a field that can not be changed anymore, this entry can be changed after deleting all results with **Clear Areas**. This resets the work table to the situation before processing so that all fields can be edited again. After that the batch processing has to be performed again.*

- **Save the batch-file:** If the work table is complete and correct you can save it and in this way create the batch-file. Do this by using **File | Save As...** Make sure that you save it into the correct location: **the batch-file must be saved into the directory that contains the files** to be quantified!

To re-open a batch-file use the corresponding icons  or  or **File | Open...** (X in fig. 15).

Note *After opening a file the work table sometimes is not correctly been displayed. You then have to force the program to actualize the screen, for example by toggling between the „Nested“- and „Work Table“-view (fig. 1).*

2.1.3 Step 2: Create a method

→ By clicking on **Method | Edit...** or the -button you start a kind of a wizard which will guide you through the setup of the method.

In the first window (fig. 4) you have to define the default injection volume that was used during acquisition and a limit value for the column „accuracy“. All values which exceed this default after processing will be displayed in red color, to allow for quick access to those integrations where obviously problems have occurred and which should be checked whether for example a manual reintegration is necessary.

→ Change the default injection volume to „10“.

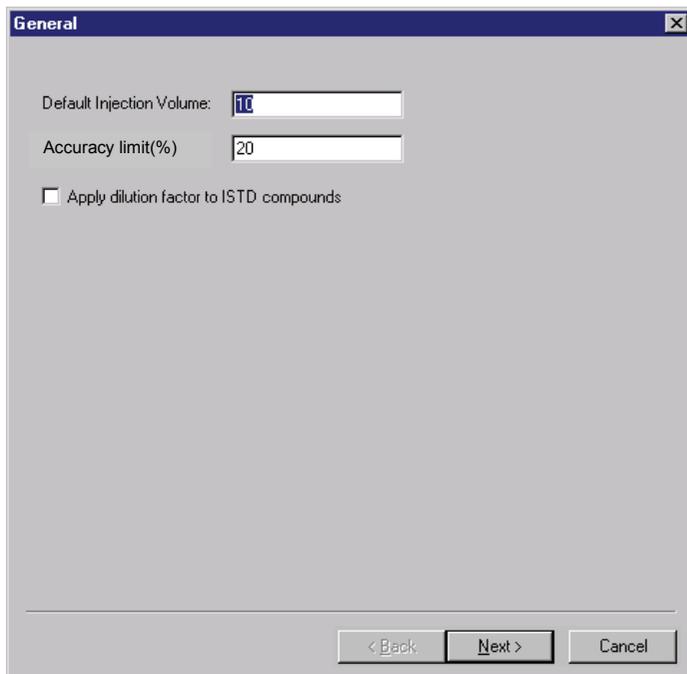


Figure 5 Method editor, part 1

In the next window (**fig. 5**) you have to define the chromatographic traces of the compounds to be quantified and their internal standards. The traces are defined in the same way as in DataAnalysis. To define them you have to know the MS details of the analysis.

In this example a target analysis was performed: Alprazolam and its d5-derivative both form [M

+H]⁺-ions (m/z = 309 and 314) which give two specific fragment ions in MS/MS experiments (see table). During the chromatographic run alternating MS/MS experiments for both compound ions are performed and the fragments are scanned and used for quantitative analysis.

	Alprazolam d0	Alprazolam d5
compound ion	[M+H] ⁺ , m/z = 309	[M+H] ⁺ , m/z = 314
fragment ions	m/z = 274, 281	m/z = 279, 286

a) Definition of the chromatographic trace of the target compound:

- In the field „Type“ choose „Extracted Ion Chromatogram“ from the drop down field.
- Choose „MSn“ as „Filter“ , enter „309“ into „of“ and „274+281“ (or „274; 281“) into „Masses“.

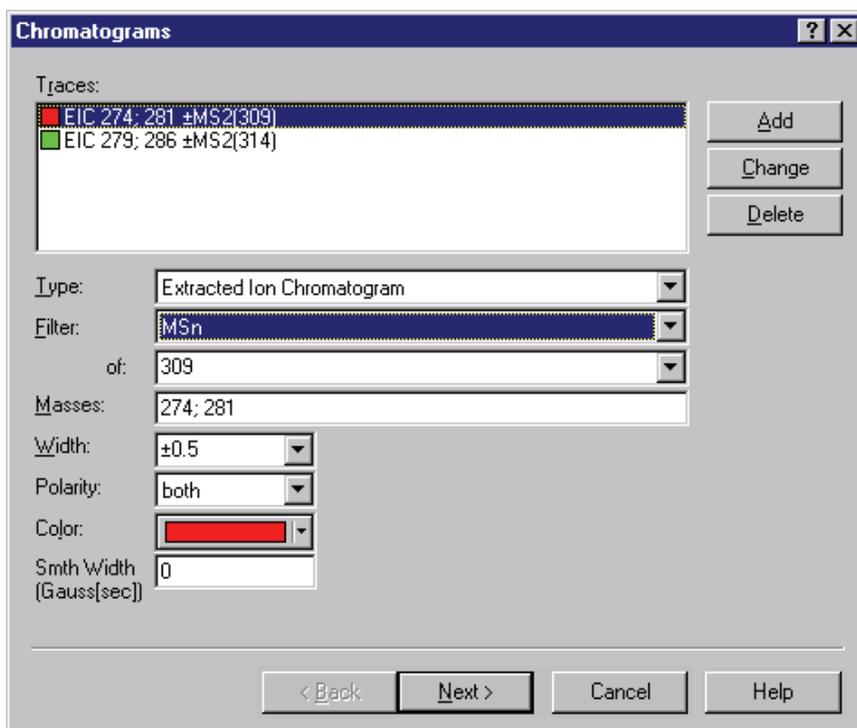


Figure 6 Method editor, part 2; Definition of chromatogram traces

Normally it is not necessary to change the „Width“, but in the case of mass shifts during analysis this width can be adjusted. In this example it also is not necessary to choose a „Polarity“, but in the case that chromatograms were acquired in „alternating mode“ (defined in EsquireControl: alternating scans in positive and negative ionization mode) you have to choose the right polarity for the trace definition.

If you want, you can choose a „Color“ for every trace, furthermore if the trace is very noisy it is possible to perform a smoothing by applying an appropriate smoothing width (given in seconds).

→ Click on **Add** to complete the trace definition.

b) Definition of the chromatographic trace of the internal standard:

- In the field „Type“ choose „Extracted Ion Chromatogram“ from the drop down field.
- Choose „MS/MS“ as „Filter“ , enter „314“ into „of“ and „279+286“ (or „279; 286“) into „Masses“.
- Click on **Add** and **Next** to complete the trace definition.

In the third window (**fig. 6**) further informations for the internal standard are to be given:

- Enter a name of choice, for example „Alprazolam d5“, into „Name“
- Choose the corresponding ion trace from the drop down field in „Chrom.“ (MS/MS 314).
- Give the retention time of the compound (in this case 1.2 min) and the width of the time window (in min) in which the software has to search for the peak (0.5).
- Enter „10“ into „Default Concentration“ (same concentration of ISTD is used for all samples) and „pg/μl“ into „Concentration units“.
- Click on **Add** and **Next**.

ISTD Compounds

Alprazolam D5

Add

Change

Delete

Name: Alprazolam D5

CAS No.:

Chrom.: EIC 279; 286 ±MS2(314)

Retention time [min]: 1.2 Window [min]: 0.5

Default Concentration: 10

Concentration units: pg/µl

< Back Next > Cancel Help

Figure 7 Method editor, part 3; Definition of ISTD

In the next window (**fig. 7**) define the target compound in the same way like the ISTD. Additionally the concentrations of calibration solutions have to be given:

- Enter a name of choice, for example „Alprazolam d0“, into „Name“
- Choose the corresponding ion trace from the drop down field in „Chrom.“ (MS/MS 309).
- Choose the corresponding ISTD compound from the drop down field in „ISTD“.
- Give the retention time of the compound (in this case 1.2 min) and the width of the time window (in min) in which the software has to search for the peak (0.5).
- Enter „pg/µl“ into „Concentration units“.
- To define the concentration values corresponding to the concentration levels given in the work table click on the „new“-icon (blue circle in **fig. 7**), enter the level ID and the concentration value separated by a „;“. Finish with **Enter** and repeat for all levels (values for example 1 see **fig. 7**).
- Click on **Add** and **Next**.

Note *In case of a multi-component quantitation and if you use more than one ISTD you also have to select the ISTD for each individual compound from the drop down field „ISTD“.*

Target Compounds

Target Compounds

Alprazolam D0

Add

Change

Delete

Name: Alprazolam D0

CAS No.:

Chrom.: EIC 274; 281 ±MS2(309)

ISTD: Alprazolam D5

Retention time [min]: 1.2 Window (min): 0.5

Concentration units: pg/µl

Standard conc.

1;2.000000
2;10.000000
3;20.000000
4;100.000000

Note: Entry must be:
"Cal.Level";"Conc."

< Back Next > Cancel Help

Figure 8 Method editor, part 4; Definition of target compounds

In the last window (**fig. 8**) the integration parameters can be adjusted. Normally the default values already allow for good integration results, but if necessary the parameters can be given independently for each individual chromatographic trace. This will for example be very helpful if there are peaks within the same chromatogram which differ a lot in their width or intensity. With only one set of integration parameters it is quite often not possible to integrate all peaks in a correct way. Optimizing the integration parameters then will minimize the need for manual integration of peaks after processing.

See DataAnalysis User's and Reference Manuals for a detailed description of the different parameters.

Click on **Finish** to close the method editor. The method can be saved with **Method | Save as...**

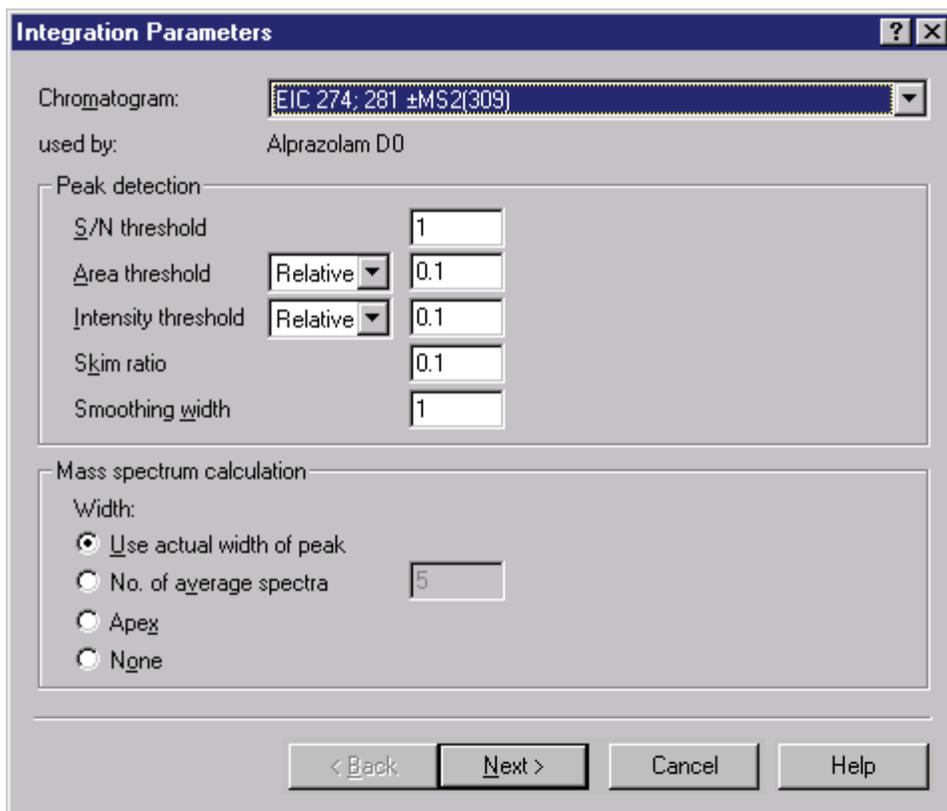


Figure 9 Method editor, part 5; Definition of integration parameters

2.1.4 Step 3: Do the quantitation and review the results

→ Start the quantitation with the button  Process.

QuantAnalysis will first do an evaluation and will give an error message if important informations are missing. After the evaluation the data files are loaded and analyzed. A blue bar at the bottom of the screen shows the progress of this process. After finishing all calculations the results are updated into the work table. The whole process normally takes only a few seconds. It is not necessary to save the batch with the results manually, this is automatically done during data analysis.

In the „**Nested**“-View simultaneously the calibration curve of the target compound and one of the corresponding peaks and its mass spectrum are displayed. If necessary adjust the relative size of the windows by dragging the separating lines between the „**Chromatogram/Spectrum**“- and the „**Calibration**“-window (red arrow in **fig. 9**).

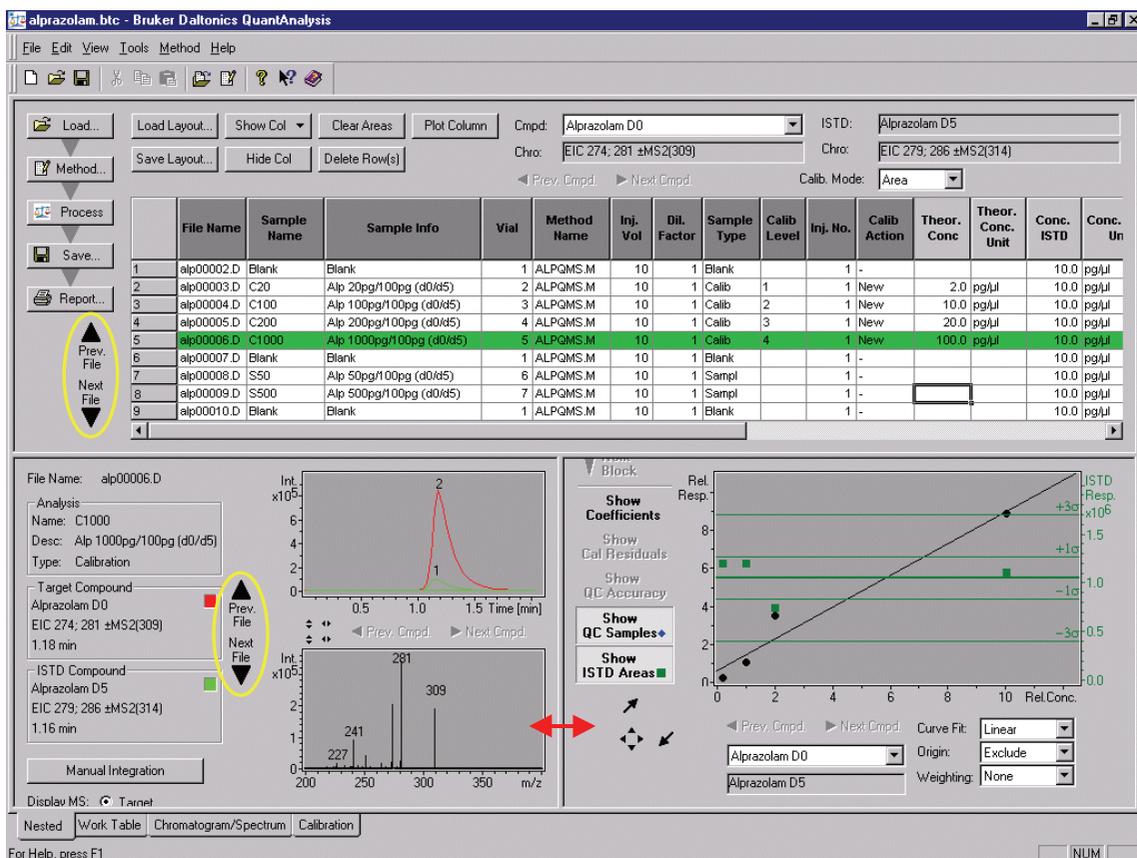


Figure 10 „Nested view“ after processing

One of the lines in the work table will be **highlighted in dark green**. This highlighting shows that the corresponding file is the „active“ one, this means that the displayed peak/spectrum belongs to it. To see the chromatograms/spectra of other files (same compound) you can use the buttons **Prev. File** and **Next File** either in the work table or in the Chromatogram/Spectrum -window (yellow ellipse in **fig. 9**).

If a peak of a compound could not be found during the integration process, the corresponding fields in the work table are set to zero. In the case that many fields remain empty please check your method again, if necessary correct it or adjust the detect parameters and restart the quantitation. The program will reprocess the whole batch of files, reprocessing of single files is not possible.

If only some peaks are not detected they can be integrated by manual integration (see manual integration on page 24).

- Adjust the scaling of the chromatograms to your needs. Activate the buttons marked by the blue circles to reset the scaling to „100%“ (or double-click on the scale) if you want to see all displays in full scale or deactivate them to get an „absolute“ scaling. The scales can be shifted and/or expanded by grabbing them with the left/right mouse button.
- To switch the mass spectrum display between target compound and ISTD use the **checkboxes** in the Chromatogram/Spectrum -window (green circle in **fig. 10**).

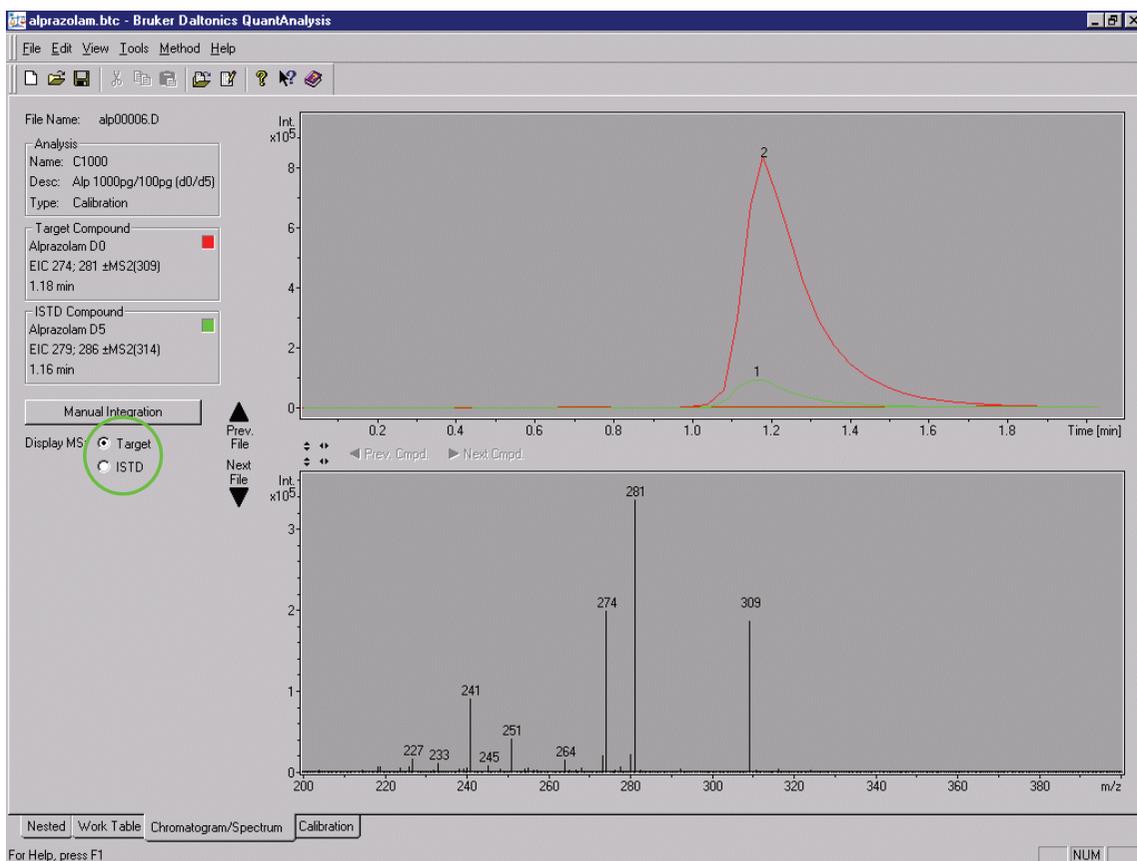


Figure 11 Chromatogram/Spectrum view

In the „Calibration“-window you can choose between different **kinds of calibration curves**, decide how the **origin** is handled and define a **weighting**. Furthermore you can **activate or deactivate each individual calibration point**: If you move the mouse near to a calibration point, the cursor changes and shows either a cross or a little hook

depending on the actual situation. Additionally a little window will appear for a few seconds displaying some information about the calibration point (calibration level, accuracy, theoretical concentration; **fig. 11**). If a point is disabled it will be displayed as an open circle instead of a black point. The effect of any change in the settings of this window will be instantaneously recalculated and displayed both here and in the work table.

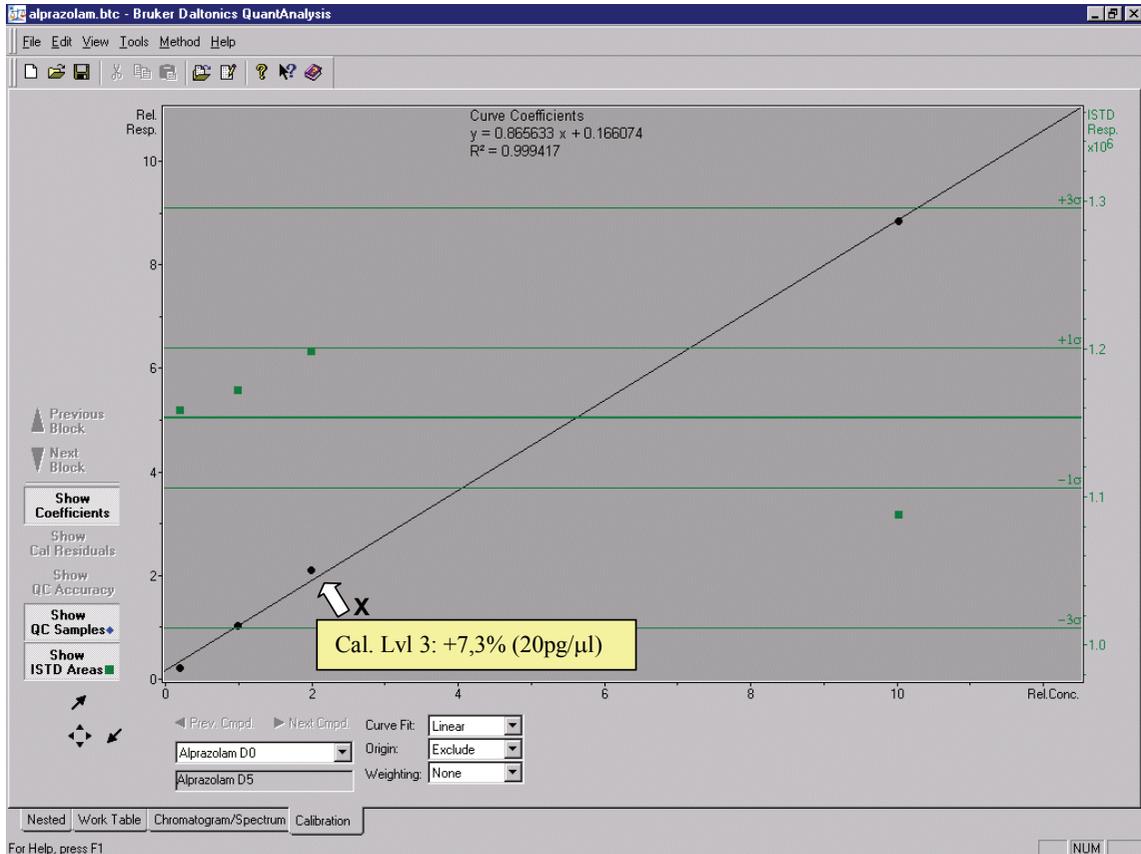


Figure 12 Calibration view

In the case of a quantitation with internal standard the integration results for the internal standard can be displayed by activating the switch **Show ISTD areas** (**fig. 11**). A green line in the middle of the window marks the averaged integration result, additional green lines denote the limits of single and triple standard deviation from this value.

→ To display the curve coefficients of the calibration curve activate the switch **Show Coefficients**. Scaling of the curve can be done in the same way as for the chromatograms and mass spectrums.

If you have defined samples as QC-type they also can be displayed in the calibration curve. Just activate the button **Show QC Samples**. However, information about them will not yet be displayed by moving the mouse-cursor next to such a point.

→ Check for misintegrated peaks. Give especially attention to chromatograms of the type „sample“ and correct the integration where necessary in manual integration mode: Activate **Manual Integration** (blue ellipse in **fig. 12**) and draw the appearing gray handles (yellow circle in **fig. 13**) to an appropriate position (see **fig. 12** and **fig. 13**). Make sure that the corresponding chromatographic trace is active (green circle in **fig. 12**).

The results are instantaneously updated in the other windows. In the work table peak areas which are obtained from manual integration are given in blue color (**fig. 14**).

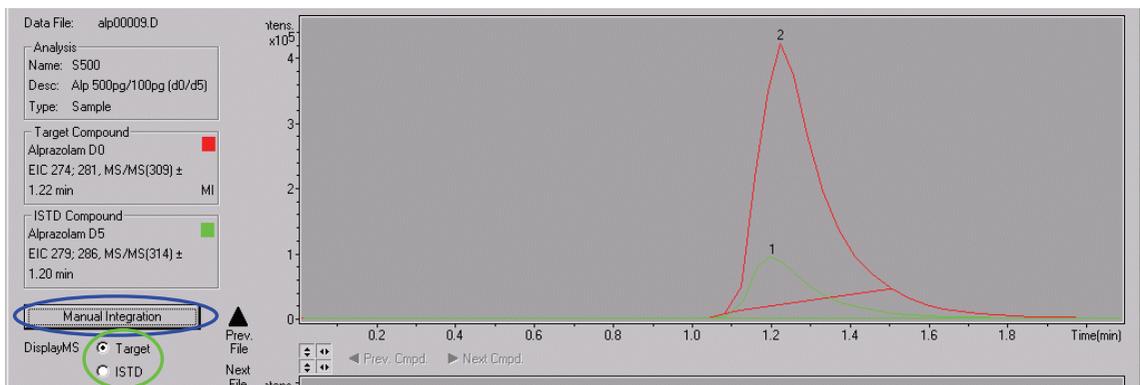


Figure 13 Starting of manual integration mode

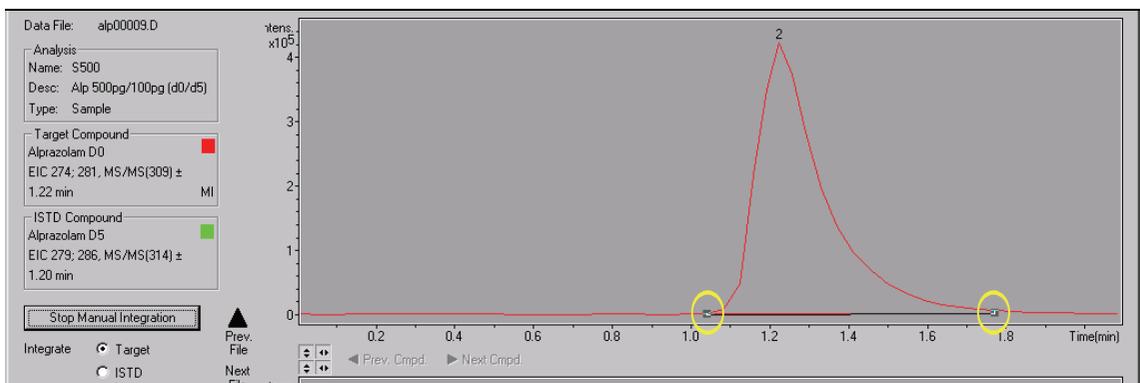


Figure 14 Manual integration mode running

	Calib Level	Inj. No.	Calib Action	Theor. Conc.	Theor. Conc. Unit	Conc. ISTD	Conc. ISTD Unit	RT Target Cmpd	Area Target Cmpd	Area ISTD Cmpd	Ratio Area Target/ISTD	Calc. Conc.	Calc. Conc. Unit	Accuracy
1		1	-			10.0	pg/µl	0.75	322			0.0	pg/µl	
2	1		New	2.0	pg/µl	10.0	pg/µl	1.26	232453	1157386	0.20084	0.4	pg/µl	20.1
3	2		New	10.0	pg/µl	10.0	pg/µl	1.23	1193707	1170482	1.01984	9.9	pg/µl	98.6
4	3		New	20.0	pg/µl	10.0	pg/µl	1.20	2490156	1197069	2.08018	22.1	pg/µl	110.6
5	4		New	100.0	pg/µl	10.0	pg/µl	1.18	9553828	1086923	8.78979	99.6	pg/µl	99.6
6		1	-			10.0	pg/µl					0.0	pg/µl	
7		1	-			10.0	pg/µl	1.22	593464	1292763	0.45907	3.4	pg/µl	
8		1	-			10.0	pg/µl	1.22	4974383	1082997	4.59316	51.1	pg/µl	
9		1	-			10.0	pg/µl					0.0	pg/µl	

Figure 15 Identification of manually (re)integrated peaks

2.1.5 step 4: Report

For reporting, there are two possibilities available:

- 1) **Export of the worktable content** as a file of type “**comma separated value**” (*.csv). This file can be opened for example with excel for further analysis of the results.

It can be created by using the button  ← Export... and can be useful for example for calculation of quantification statistics, which are not yet available within QuantAnalysis.

- 2) **Printing of the results:** Printed reports can be created via the button  Report... In the current version of QuantAnalysis two report layouts for printing are available from the corresponding drop-down field in the printing dialog:

- a) QuantAnalysis Summary Report
- b) QuantAnalysis Summary (ISTD) Report

Both layouts differ only in the columns, which are printed from the result part of the worktable. Whereas the first report prints only the compound relevant columns (AreaTargetCmpd, Calc.Conc., Accuracy), the printouts for the second report shows additionally the results for the internal standard. This report therefore is meant only for quantifications with internal standards.

2.2 Example 2: Quantitation of a set of data files obtained individually

2.2.1 Remarks

This example shows a **multi-component quantitation** with **external calibration**.

Target compounds: three carbamates:

Propamocarb („**PCB**“), MW 188

Carbendazim („**CBZ**“), MW 191

Pirimicarb („**PRC**“), MW 238

The series consists of eight **individually acquired data files**. Four standard solutions containing each compound at a concentration level of 0.1, 1, 10 and 100 pg/μl were used with an injection volume of 10 μl. Three additional runs using only an injection volume of 5 μl were used to get supplementary calibration points (simulating concentration levels of 0.5, 5 and 50 pg/μl). The solution containing 100 pg/μl of each carbamate functioned as „unknown” sample (injection volume 3 μl).

2.2.2 Step 1: Generation of the batch file

→ Switch to the full-screen display for the work table (red ellipse in **fig. 1**).

In contrast to example 1 it is in this case not possible to import a part of the work table automatically for all data files. You have to fill in all informations manually:

Note *Entries can be edited with Cut/Copy/Paste and with an „Excel like fill”: select a field that you want to copy multiple times by clicking on it and move the cursor to the lower right corner of the field until the cursor changes to a „+“. Now you can fill out a column just by dragging the mouse (left mouse-button kept pressed). Within the field „Vial“ the number will be incremented and the same is the case if the field „FileName“ contains a number. This „Excel like fill” is only active before the batch was processed.*

→ **Enter the names of the individual files (fig. 15)**. Make sure that all data files, which are needed, are copied into one directory. You can **not** open these files by an icon or by **File | Open**, this command can only be applied to batch-files.

Note *The file names must be entered together with the file extension “.d” (fig. 15)!*

→ Give all other informations needed for the batch processing: fill out the work table according to **fig. 16**. Adjust layout for external calibration (see step 1 of example 1; hide columns with ISTD data, adjust column widths).

Note *If you want to do an external calibration and you have done alternating measurements of unknown and calibration samples you can not enter the files in the same order as you have run them. In the moment you have to enter first all samples of the type „calibration“ because the program will calculate an additional calibration curve if it finds a second block of calibration samples after there was a type „sample“! It is not possible then to enter all calibration points into only one calibration curve. You only can toggle between the different calibration curves which are defined by different blocks (buttons „previous block“ and „next block“ in the calibration view). Please consider this fact in the setup of a sequence! If you have to do alternating runs of unknown and calibration samples (it is in fact recommended that you do so in the case of an external calibration) you can import the sequence file, but you then have to sort the rows in the work table: mark a row by clicking on the row number and drag the line to an appropriate position (a red line appears at the position if insertion; make sure that the mouse cursor remains above the column of row numbers).*

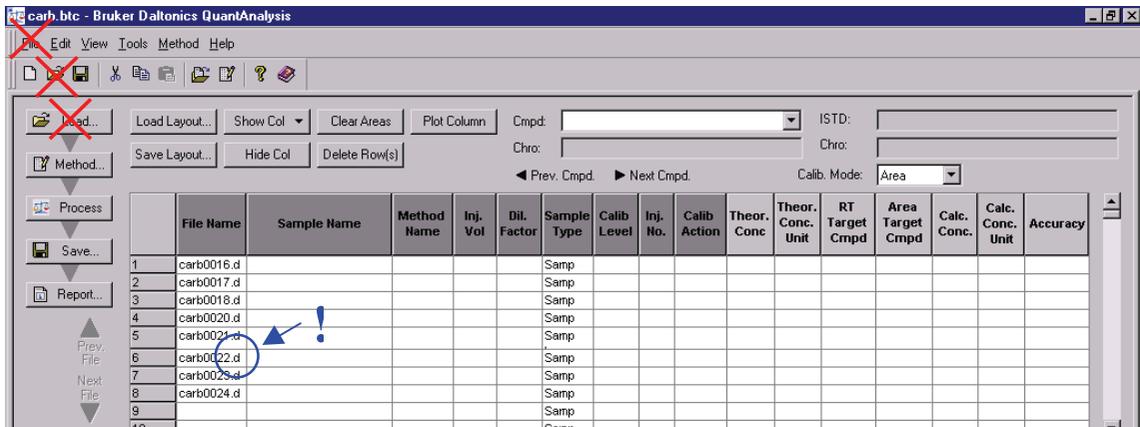
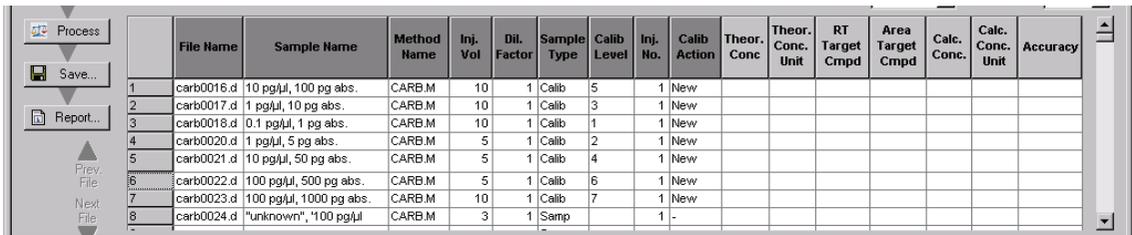


Figure 16 Manual entering of file names



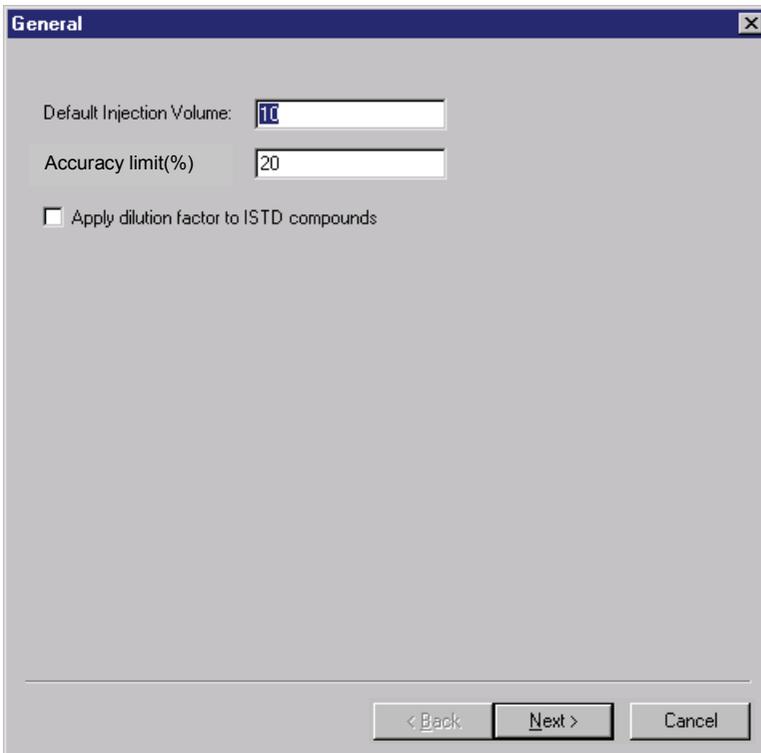
	File Name	Sample Name	Method Name	Inj. Vol	Dil. Factor	Sample Type	Calib Level	Inj. No.	Calib Action	Theor. Conc.	Theor. Conc. Unit	RT Target Cmpd	Area Target Cmpd	Calc. Conc.	Calc. Conc. Unit	Accuracy
1	carb0016.d	10 pg/ul, 100 pg abs.	CARB.M	10	1	Calib	5	1	New							
2	carb0017.d	1 pg/ul, 10 pg abs.	CARB.M	10	1	Calib	3	1	New							
3	carb0018.d	0.1 pg/ul, 1 pg abs.	CARB.M	10	1	Calib	1	1	New							
4	carb0020.d	1 pg/ul, 5 pg abs.	CARB.M	5	1	Calib	2	1	New							
5	carb0021.d	10 pg/ul, 50 pg abs.	CARB.M	5	1	Calib	4	1	New							
6	carb0022.d	100 pg/ul, 500 pg abs.	CARB.M	5	1	Calib	6	1	New							
7	carb0023.d	100 pg/ul, 1000 pg abs.	CARB.M	10	1	Calib	7	1	New							
8	carb0024.d	"unknown", "100 pg/ul	CARB.M	3	1	Samp	-	1	-							

Figure 17 Completed work table for example 2

2.2.3 Step 2: Create a method

Method setup is very similar to that of example 1, therefore please see also step 2 of example 1.

- By clicking on **Method | Edit...** or on  you start a kind of a wizard (fig. 17) which will guide you through the setup of the method.
- Change the default injection volume to „10“.



General

Default Injection Volume:

Accuracy limit(%)

Apply dilution factor to ISTD compounds

< Back Next > Cancel

Figure 18 Method editor, part 1

In the next window (**fig. 18**) you have to define the chromatographic traces of the compounds to be quantified.

In this example a target analysis with time table was performed:

0,00 – 1,80 min:	MS/MS 189	([M+H] ⁺ of PCB, retention time: 1,4 min)
1,85 – 3,20 min:	MS/MS 192	([M+H] ⁺ of CBZ, retention time: 2,1 min)
3,30 – end:	MS/MS 239	([M+H] ⁺ of PCR, retention time: 4,0 min)

Ion traces for quantitation: PCB: sum trace of m/z = 102+144
CBZ: ion trace of m/z = 160
PCR: sum trace of m/z = 166+182+195

Definition of the chromatographic **traces of the target compounds**:

- In the field „Type“ choose „Extracted Ion Chromatogram“ from the drop down field.
- Choose „MSn“ as „Filter“ , enter „189“ into „of“ and „102+144“ (or „102; 144“) into „Masses“.
- Click on **Add** to complete the trace definition for PCB and repeat these steps with the corresponding values for the traces of CBZ and PCR.
- Click on **Next** and skip the next window with **Next** since no ISTD was used.

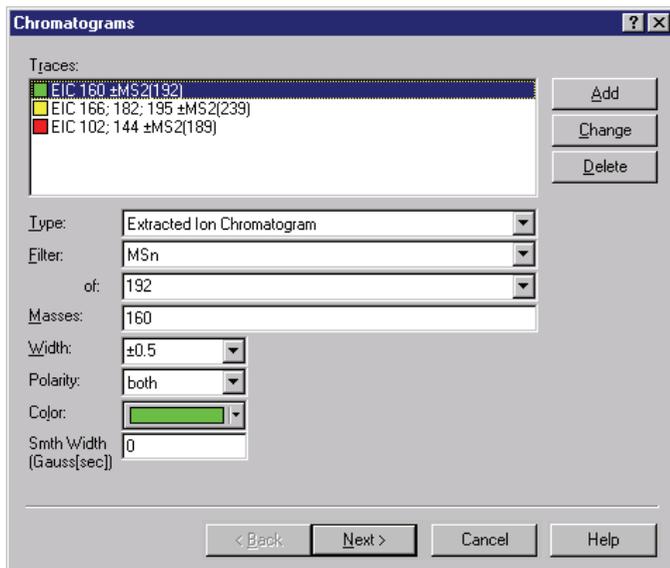


Figure 19 Method editor, part 2; Definition of chromatogram traces

Define the three target compounds (**fig. 19**) and additionally the concentrations of their calibration solutions:

- Enter a name of choice, for example „PCB“, into „Name“
- Choose the corresponding ion trace from the drop down field in „Chrom.“ (MS/MS 189).
- Give the retention time of the compound (in this case 1.4 min) and the width of the time window (in min) in which the software has to search for the peak (0.5).
- Enter „pg/μl“ into „Concentration units“.
- To define the concentration values corresponding to the concentration levels given in the work table click on the „new“-icon (blue circle in **fig. 19**), enter the level ID and the concentration value separated by a „:“. Finish with **Enter** and repeat for all levels (values for example 2 see **fig. 19**).
- Click on **Add** and repeat these steps for CBZ and PCR. Since the concentrations of all compounds are always the same for the same sample you do not need to type in the level definitions again.
- Click on **Next**.

For details about the last window of method setup (integration parameters) see example 1.

Target Compounds

Target Compounds

- CBZ
- PCB
- PCR

Name: CBZ

CAS No.:

Chrom.: EIC 160 ±MS2(192)

ISTD: <None>

Retention time [min]: 2.1 Window [min]: 0.5

Concentration units: pg/μl

Standard conc.

- 1;0.100000
- 2;1.000000
- 3;1.000000
- 4;10.000000
- 5;10.000000
- 6;100.000000
- 7;100.000000

Note: Entry must be: "Cal.Level";"Conc."

< Back Next > Cancel Help

Figure 20 Method editor, part 4; Definition of target compounds

2.2.4 Step 3: Do the quantitation and review the results

→ Start the quantitation with the button



See step 3 of example 1 for details about the batch processing procedure and general functions of the different views.

In this example you have to check the results for several independent components: By using the buttons **Prev. Cmpd.** and **Next Cmpd.** (blue ellipse in **fig. 20**) you can toggle between the different compounds. Which compound is the „active“ one can be seen from the entry in the **drop-down field** above/below these buttons. This field also allows direct access to a special compound if the quantitation contains many compounds.

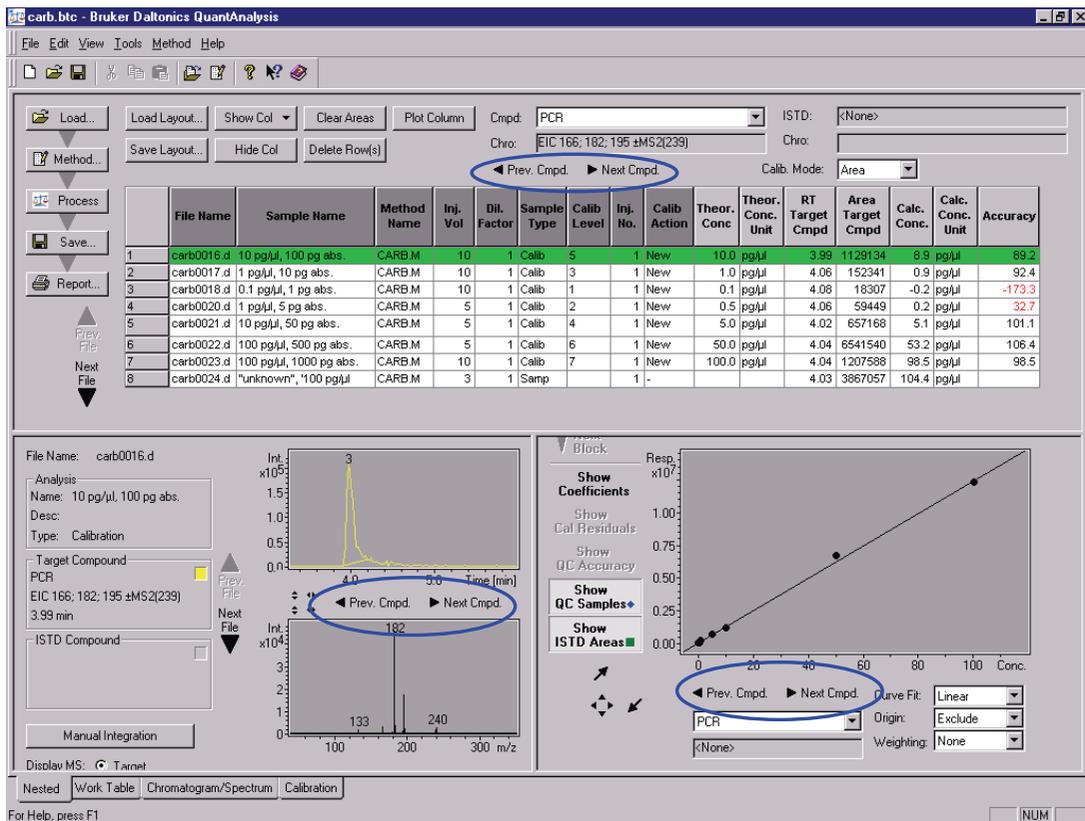


Figure 21 „Nested view“ for compound PCR after batch processing

2.2.5 Step 4: Report

Please see chapter 2.1.5 for reporting options!

3 Additional remarks

3.1 Multiple injections of a sample

The program identifies multiple injections from the entries in the columns „Calib Level“ and „Inj. No.“. If you import a Agilent ChemStation sequence QuantAnalysis (in the actual version) only reads the total number of injections for a sample and therefore sets all fields „Inj. No.“ which belong to this sample to for example „3“. You have to change these fields manually to „1“, „2“, „3“ for the corresponding data files, if you would like to label each chromatogram file with the corresponding number of injection.

During batch processing QuantAnalysis first calculates the average value for these injections and uses this value to calculate the calibration curve. The average value is displayed in the calibration view as a green point. To disable it you have to disable all calibration points of this level individually.

3.2 Quality control samples

To validate a calibration curve, quality control samples can be used during acquisition. QuantAnalysis can handle these samples as type „QC“ and displays them as blue rhombus.

Fig. 21 shows the batch of example 2 processed with data file carb0022.d defined as type „QC“. Please note that in this batch the order of data files is changed compared to **fig. 16**.

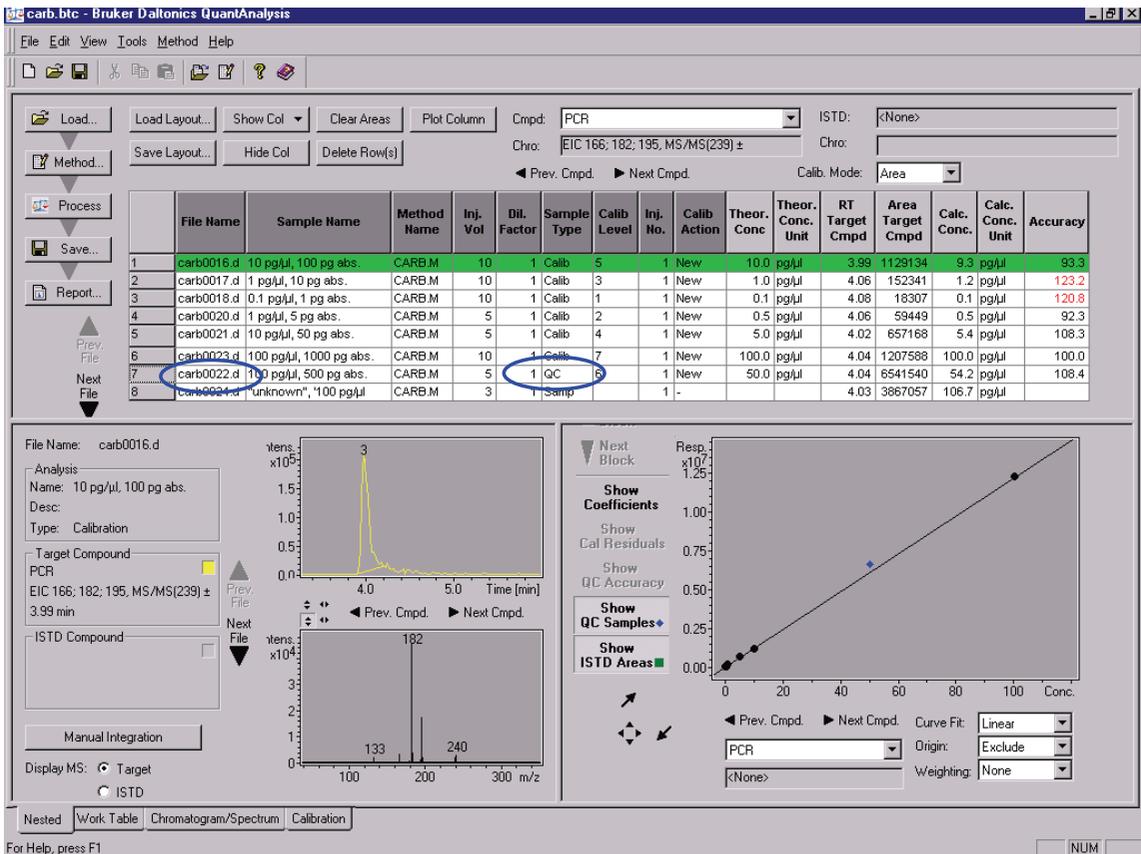


Figure 22 Calibration curve including a QC-type sample

Please compare also with fig. 22 and 23, where the same QC data file is located in between the calibration data files. In this case QuantAnalysis creates **two different calibration curves**. One contains the calibration levels „5“, „3“, and „1“, the other contains „2“, „4“ and „7“. With **Next Block** and **Previous Block** you can toggle between both curves (red circles in fig. 22 and 23). This feature can be used to quantify different sample series with for example different matrices but the same target compounds in one sequence.

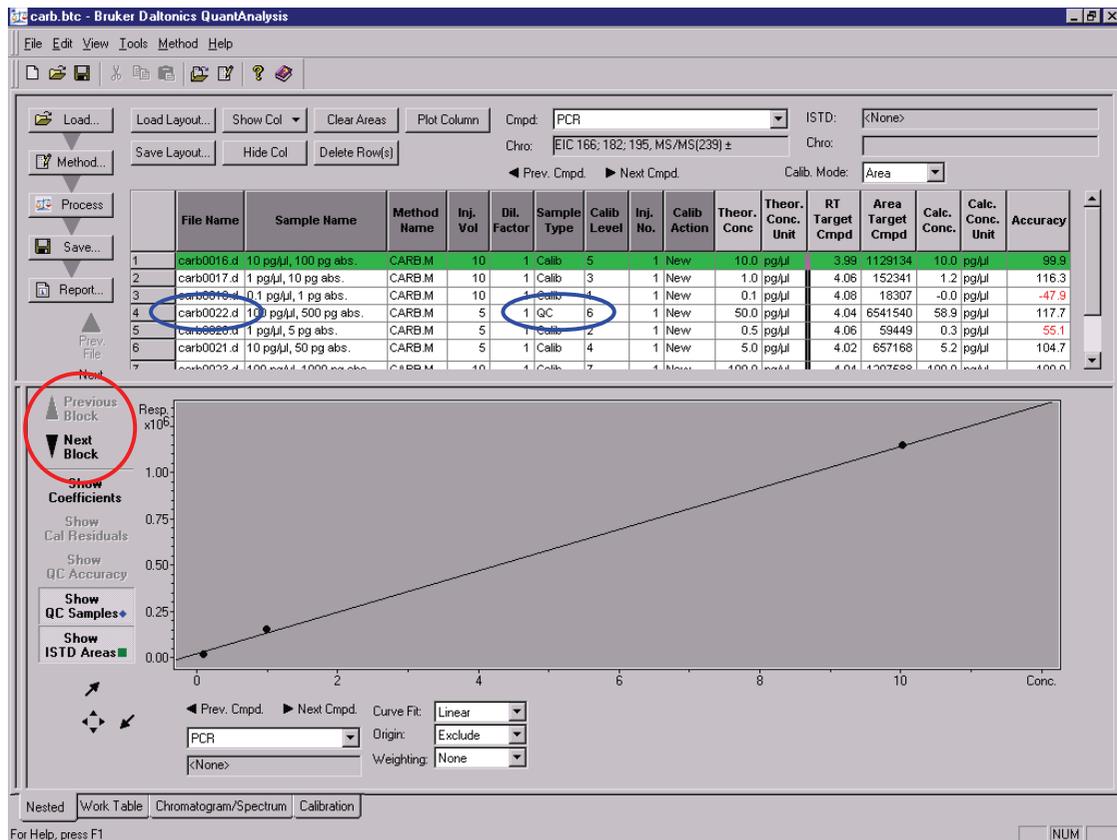


Figure 23 Generation of multiple calibration curves within one batch: Curve 1

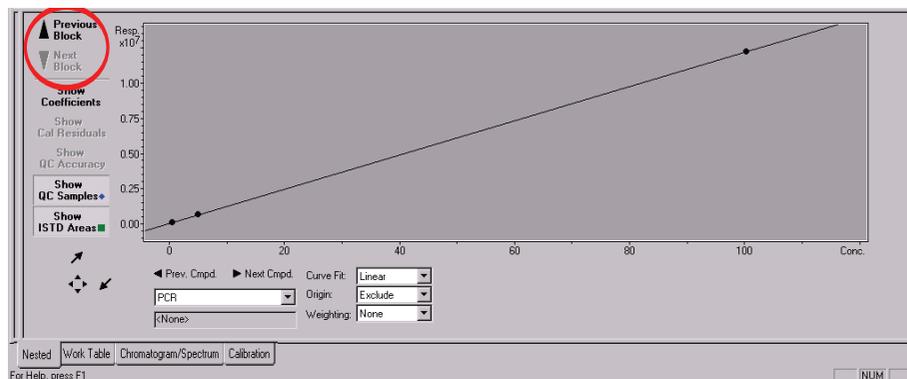


Figure 24 Generation of multiple calibration curves within one batch: Curve 2