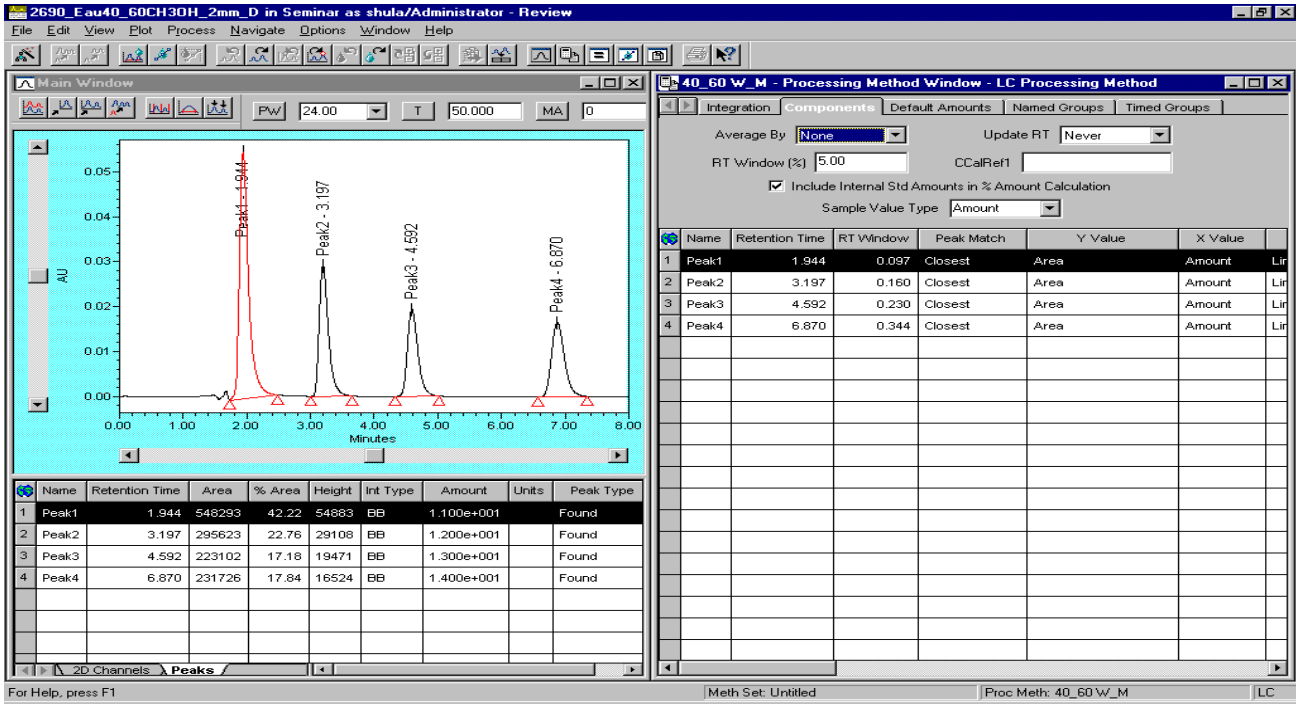
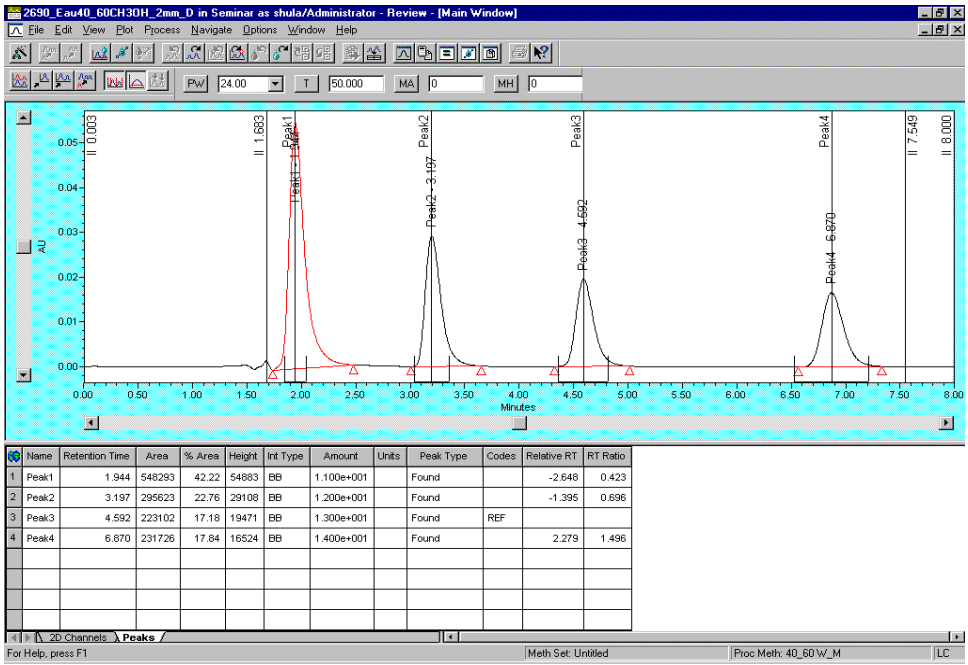
Processing of Data - Basic Quantitative Calculations

First stage is adjusting the integration events (See the integration events section) and the components in the processing method by opening a channel in the *Review* window and opening the specific *Processing Method*.

If the components are not defined yet in the *Processing method*, the following should be done: in the *Review* window tile the *Main window* with the *Processing method* , select *Process -> Integrate*, go to the *Components* window: To fill in the components use *Option -> Fill from Results*. The software automatically enters them. Once they are in the component table their names can be edited and other parameters in the Component Table can be entered (RT windows, Int Std as specified later on). Then select *Process -> Integrate-> Calibrate* to get the results in the left window. (It is easier to tile the two windows, the Main and the Processing Method's window in order to edit the method and see the results simultaneously). Remember to save the method.



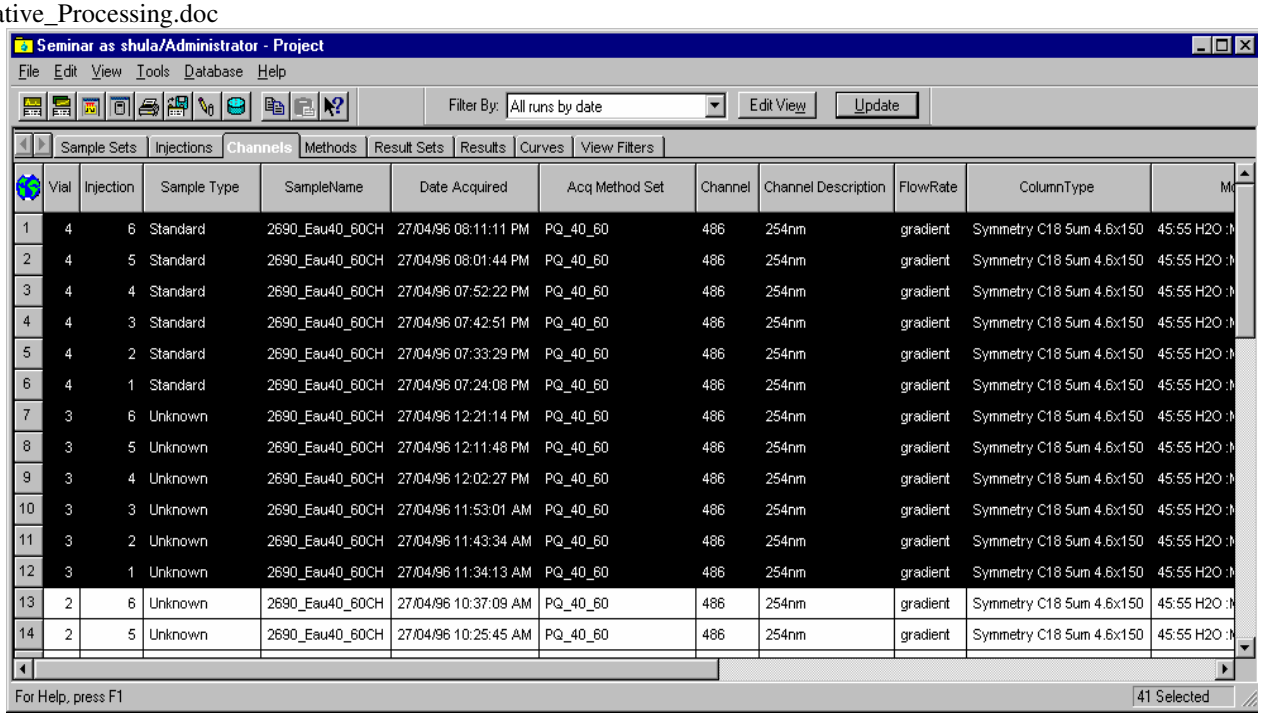
Once the components are assigned and registered, the peaks names appear in the chromatogram. Use the *Option-> Show Events* and *Show Components* to be able to see them graphically on the screen.



The Component table includes the following columns:

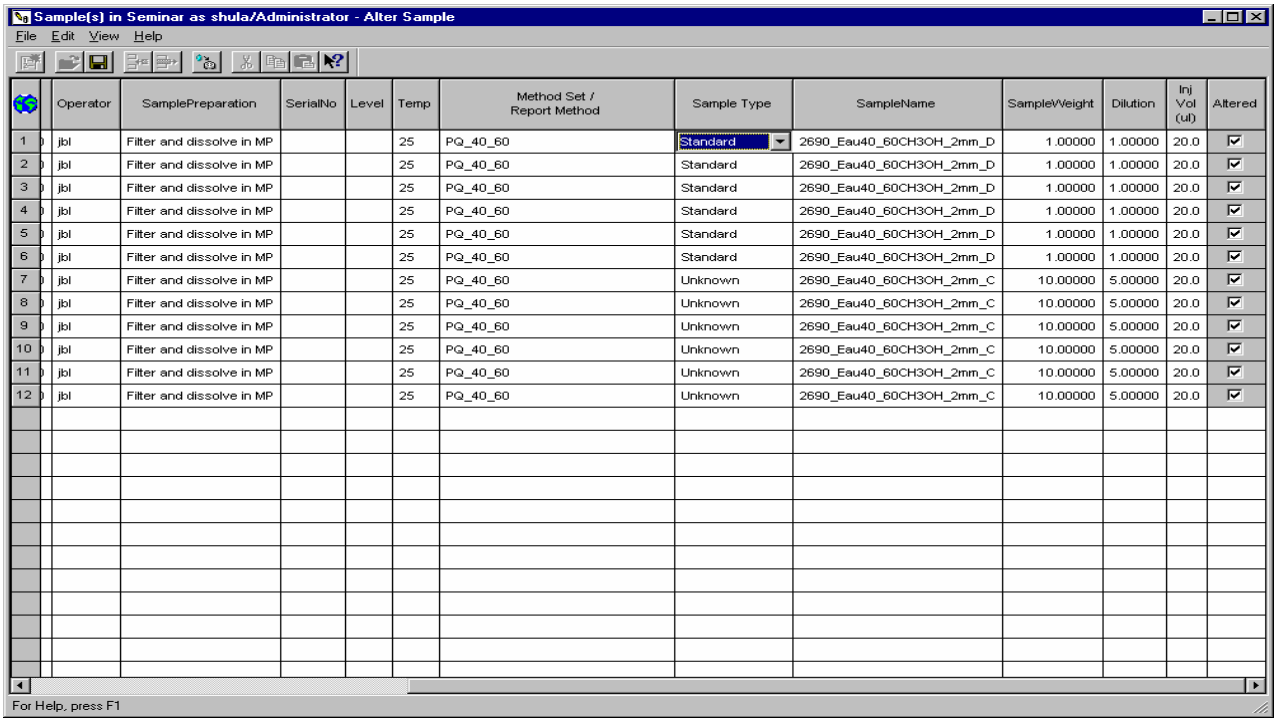
Name	**Identifies the component**
Retention Time	Assigns the RT +/- the RT window
RT Window	The range within which the peak is identifies
Peak Match	Type of match to the RT (Closest, highest, second, etc.)
Y Value	Instead of just the area (% area, height, etc.)
X Value	Amount or Concentration
Fit	Linear, cubic, linear through zero, others.
Weighting	Points in the calibration curve can be weighted (1/x, logx etc.) to emphasize portions of the curve
Internal Std	The component's name entered here will serve as the internal standard.
RT Reference	The component's name entered here will serve as the RT reference for RRT.
Rel Resol Reference (SST)	The component's name entered here will serve for the relative Rs.
Curve Reference	The calibration curve of the component's name entered here will be used.
Relative Response	The component's name entered here will serve for the relative response..
Must	Yes or No. If it is a Must peak and it is missing, the result won't be produced.
Default Pk	If it is a default peak, its calibration curve will be used to quantify unknowns.
Default Pk Start & End	Sets the range in the chromatogram in which unknowns will by quantified using this specific default peak.
Type	Single, Timed or Named group.
CCompRef1, CCompRef2, CCompRef3	Used for inter-peak calculations
CConst1, CConst2	Used for individual peaks calculations, constant parameters can be introduced here (such as label claim, Dissolution chamber's volume etc.).
Default Units	The reported units of the final result.

Once the integration events were set appropriately, the next step is to enter the information related to the channels, the chromatographic conditions and the quantitative parameters. This is done at the *Channel* window, using the tool: *Alter Sample*.



	Vial	Injection	Sample Type	SampleName	Date Acquired	Acq Method Set	Channel	Channel Description	FlowRate	ColumnType	M
1	4	6	Standard	2690_Eau40_60CH	27/04/96 08:11:11 PM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
2	4	5	Standard	2690_Eau40_60CH	27/04/96 08:01:44 PM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
3	4	4	Standard	2690_Eau40_60CH	27/04/96 07:52:22 PM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
4	4	3	Standard	2690_Eau40_60CH	27/04/96 07:42:51 PM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
5	4	2	Standard	2690_Eau40_60CH	27/04/96 07:33:29 PM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
6	4	1	Standard	2690_Eau40_60CH	27/04/96 07:24:08 PM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
7	3	6	Unknown	2690_Eau40_60CH	27/04/96 12:21:14 PM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
8	3	5	Unknown	2690_Eau40_60CH	27/04/96 12:11:48 PM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
9	3	4	Unknown	2690_Eau40_60CH	27/04/96 12:02:27 PM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
10	3	3	Unknown	2690_Eau40_60CH	27/04/96 11:53:01 AM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
11	3	2	Unknown	2690_Eau40_60CH	27/04/96 11:43:34 AM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
12	3	1	Unknown	2690_Eau40_60CH	27/04/96 11:34:13 AM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
13	2	6	Unknown	2690_Eau40_60CH	27/04/96 10:37:09 AM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M
14	2	5	Unknown	2690_Eau40_60CH	27/04/96 10:25:45 AM	PQ_40_60	486	254nm	gradient	Symmetry C18 5um 4.6x150	45:55 H2O :M

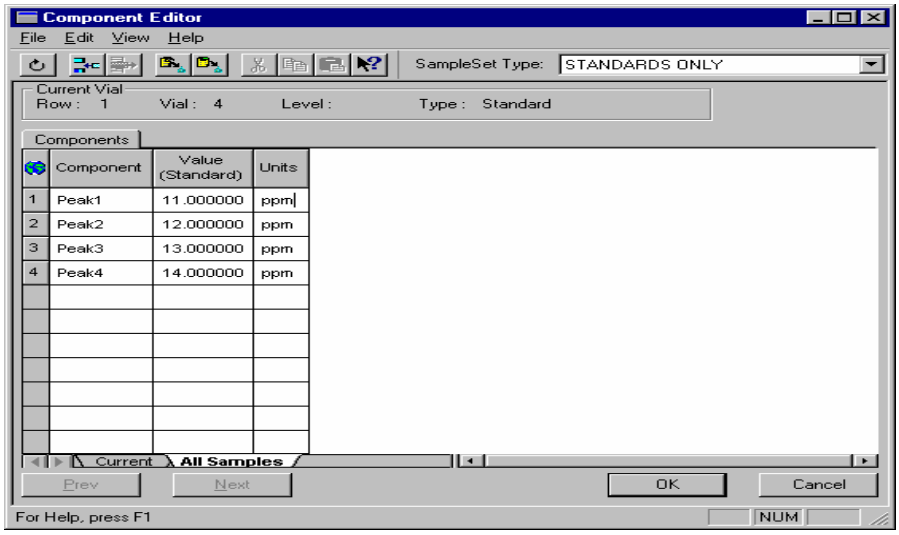
A table appears with a list of the samples. Some of the fields are editable, such as the sample name, the sample status *(Standard* or *Unknown*), text in the custom field such as *Mobile_phase* and *Column_type*, etc. The quantitative parameters are entered in the *Sample weight* and *Dilution* and other custom fields that were created for custom calculations if necessary (custom fields such as *Average Tablet Weight* etc.)



	Operator	SamplePreparation	SerialNo	Level	Temp	Method Set / Report Method	Sample Type	SampleName	SampleWeight	Dilution	Inj Vol (ul)	Altered
1	jbl	Filter and dissolve in MP			25	PQ_40_60	Standard	2690_Eau40_60CH3OH_2mm_D	1.00000	1.00000	20.0	☑
2	jbl	Filter and dissolve in MP			25	PQ_40_60	Standard	2690_Eau40_60CH3OH_2mm_D	1.00000	1.00000	20.0	☑
3	jbl	Filter and dissolve in MP			25	PQ_40_60	Standard	2690_Eau40_60CH3OH_2mm_D	1.00000	1.00000	20.0	☑
4	jbl	Filter and dissolve in MP			25	PQ_40_60	Standard	2690_Eau40_60CH3OH_2mm_D	1.00000	1.00000	20.0	☑
5	jbl	Filter and dissolve in MP			25	PQ_40_60	Standard	2690_Eau40_60CH3OH_2mm_D	1.00000	1.00000	20.0	☑
6	jbl	Filter and dissolve in MP			25	PQ_40_60	Standard	2690_Eau40_60CH3OH_2mm_D	1.00000	1.00000	20.0	☑
7	jbl	Filter and dissolve in MP			25	PQ_40_60	Unknown	2690_Eau40_60CH3OH_2mm_C	10.00000	5.00000	20.0	☑
8	jbl	Filter and dissolve in MP			25	PQ_40_60	Unknown	2690_Eau40_60CH3OH_2mm_C	10.00000	5.00000	20.0	☑
9	jbl	Filter and dissolve in MP			25	PQ_40_60	Unknown	2690_Eau40_60CH3OH_2mm_C	10.00000	5.00000	20.0	☑
10	jbl	Filter and dissolve in MP			25	PQ_40_60	Unknown	2690_Eau40_60CH3OH_2mm_C	10.00000	5.00000	20.0	☑
11	jbl	Filter and dissolve in MP			25	PQ_40_60	Unknown	2690_Eau40_60CH3OH_2mm_C	10.00000	5.00000	20.0	☑
12	jbl	Filter and dissolve in MP			25	PQ_40_60	Unknown	2690_Eau40_60CH3OH_2mm_C	10.00000	5.00000	20.0	☑

It is extremely important to set the *Sample type* (Standard or Unknown) right. A channel that is defined a *Standard* will be used for calibration, and a channel that is defined an *Unknown* will be quantified using the calibration curve in the processing method that is used for them.

Weights or Concentrations of the standards are entered in the *Amount loading table*, using *Edit -> Amounts*. The components names are inserted either manually or using: *Edit -> Copy components from process method* in the *Amounts Loading Table*. The components are inserted automatically by the software in the *Name* column.



It is essential that the components names will be identical to those in the processing method, otherwise they will not be quantified. Click on the **OK** button and in the *Alter Sample Table* Select *File->Save*. Once the amounts and weights and other parameters were introduced, it is time to process them manually in the *Review* window of the channels, before attempting batch processing to check if everything was entered OK.

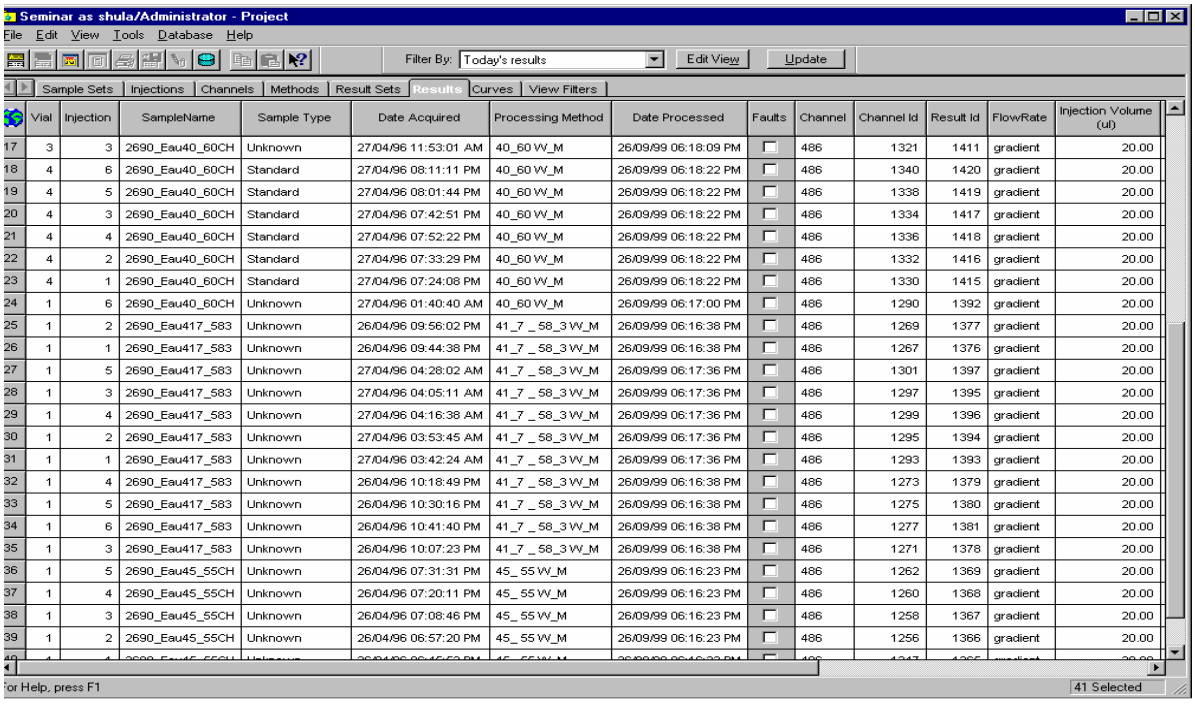
During method development the chromatograms are not routine, so it is advisable to work on them in the Review one by one and check whether the components are registered and calculated properly. It is possible to select a few channels together and review them using *Tools -> Review*, and then process them one after the other using: *Navigate -> Next 2D channel -> Integrate -> Calibrate/Quantitate* in a regular 2D work or *Navigate -> Next 3D channel* in PDA work (*Apply Method Set* is done automatically). When you see that all the results are there, exit the *Review* window and use batch processing (see below).

The quantitative results will not be performed unless the following is fulfilled:

- The component name in the *Amount Loading Table* are identical to those in the processing method.
- The analysed peak is within the RT window range and identified
- The calibration curve was not cleared before integrating the unknowns.

Once you set the components right and the Peak table shows that they were quantified properly, you can exit and use the Processing tool to product all the results at once. In the Channel View select the channels, select *Tools -> Process -> Use specified processing method* (in 2D work) or *Use specified Method set* (in 3D work). The *Process* tool does the following: *Integrate*, *Calibrate* (standards) or *Quantitate* (Unknowns), *Save result, and Save Calibration* all at once.

Since the processing is done usually manually during method development, it is advisable to prepare a special *View filter* in the results window. Use the filter: *Results today* to omit old results, by clicking "*Edit View*", and filling *=Today* in the column *Date Processed*, and save it as a filter (for example: *Results today).* It is also recommended to avoid producing unnecessary results to prevent confusion. You can delete results as you wish, since they are easily reproducible.



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File Edit View Tools Database Help

Filter By: Today's results | Edit View | Update

Sample Sets | Injections | Channels | Methods | Result Sets | Results | Curves | View Filters

	Vial	Injection	SampleName	Sample Type	Date Acquired	Processing Method	Date Processed	Faults	Channel	Channel Id	Result Id	FlowRate	Injection Volume (ul)
17	3	3	2690_Eau40_60CH	Unknown	27/04/96 11:53:01 AM	40_60 W_M	26/09/99 06:18:09 PM		486	1321	1411	gradient	20.00
18	4	6	2690_Eau40_60CH	Standard	27/04/96 08:11:11 PM	40_60 W_M	26/09/99 06:18:22 PM		486	1340	1420	gradient	20.00
19	4	5	2690_Eau40_60CH	Standard	27/04/96 08:01:44 PM	40_60 W_M	26/09/99 06:18:22 PM		486	1338	1419	gradient	20.00
20	4	3	2690_Eau40_60CH	Standard	27/04/96 07:42:51 PM	40_60 W_M	26/09/99 06:18:22 PM		486	1334	1417	gradient	20.00
21	4	4	2690_Eau40_60CH	Standard	27/04/96 07:52:22 PM	40_60 W_M	26/09/99 06:18:22 PM		486	1336	1418	gradient	20.00
22	4	2	2690_Eau40_60CH	Standard	27/04/96 07:33:29 PM	40_60 W_M	26/09/99 06:18:22 PM		486	1332	1416	gradient	20.00
23	4	1	2690_Eau40_60CH	Standard	27/04/96 07:24:08 PM	40_60 W_M	26/09/99 06:18:22 PM		486	1330	1415	gradient	20.00
24	1	6	2690_Eau40_60CH	Unknown	27/04/96 01:40:40 AM	40_60 W_M	26/09/99 06:17:00 PM		486	1290	1392	gradient	20.00
25	1	2	2690_Eau417_583	Unknown	26/04/96 09:56:02 PM	41_7 _ 58_3 W_M	26/09/99 06:16:38 PM		486	1269	1377	gradient	20.00
26	1	1	2690_Eau417_583	Unknown	26/04/96 09:44:38 PM	41_7 _ 58_3 W_M	26/09/99 06:16:38 PM		486	1267	1376	gradient	20.00
27	1	5	2690_Eau417_583	Unknown	27/04/96 04:28:02 AM	41_7 _ 58_3 W_M	26/09/99 06:17:36 PM		486	1301	1397	gradient	20.00
28	1	3	2690_Eau417_583	Unknown	27/04/96 04:05:11 AM	41_7 _ 58_3 W_M	26/09/99 06:17:36 PM		486	1297	1395	gradient	20.00
29	1	4	2690_Eau417_583	Unknown	27/04/96 04:16:38 AM	41_7 _ 58_3 W_M	26/09/99 06:17:36 PM		486	1299	1396	gradient	20.00
30	1	2	2690_Eau417_583	Unknown	27/04/96 03:53:45 AM	41_7 _ 58_3 W_M	26/09/99 06:17:36 PM		486	1295	1394	gradient	20.00
31	1	1	2690_Eau417_583	Unknown	27/04/96 03:42:24 AM	41_7 _ 58_3 W_M	26/09/99 06:17:36 PM		486	1293	1393	gradient	20.00
32	1	4	2690_Eau417_583	Unknown	26/04/96 10:18:49 PM	41_7 _ 58_3 W_M	26/09/99 06:16:38 PM		486	1273	1379	gradient	20.00
33	1	5	2690_Eau417_583	Unknown	26/04/96 10:30:16 PM	41_7 _ 58_3 W_M	26/09/99 06:16:38 PM		486	1275	1380	gradient	20.00
34	1	6	2690_Eau417_583	Unknown	26/04/96 10:41:40 PM	41_7 _ 58_3 W_M	26/09/99 06:16:38 PM		486	1277	1381	gradient	20.00
35	1	3	2690_Eau417_583	Unknown	26/04/96 10:07:23 PM	41_7 _ 58_3 W_M	26/09/99 06:16:38 PM		486	1271	1378	gradient	20.00
36	1	5	2690_Eau45_55CH	Unknown	26/04/96 07:31:31 PM	45_ 55 W_M	26/09/99 06:16:23 PM		486	1262	1369	gradient	20.00
37	1	4	2690_Eau45_55CH	Unknown	26/04/96 07:20:11 PM	45_ 55 W_M	26/09/99 06:16:23 PM		486	1260	1368	gradient	20.00
38	1	3	2690_Eau45_55CH	Unknown	26/04/96 07:08:46 PM	45_ 55 W_M	26/09/99 06:16:23 PM		486	1258	1367	gradient	20.00
39	1	2	2690_Eau45_55CH	Unknown	26/04/96 06:57:20 PM	45_ 55 W_M	26/09/99 06:16:23 PM		486	1256	1366	gradient	20.00

For Help, press F1 | 41 Selected

There are 3 sets of results in the above window from 3 different mobile phase conditions. Note that there are 5 replicates, to check the precision already at this early stage.

These sets will be selected for the reporting, using the Preview tool to see the report on the screen, or the Print tool to print it directly to the printer (when the report templates are known and established).

The calibration curve is attached to the *Processing Method* or can be found at the *Curves* window.

The basic calculation is

$$Amount = \frac{Area_{Smp} * Amount_{Std} * SampleWt_{Std} * Dil_{Smp}}{Area_{Std} * SampleWt_{Smp} * Dil_{Std}}$$

Or:

$$Amount = \frac{Area_{Smp} x Amount_{Std} x (SampleWt_{Std} / Dil_{Std})}{Area_{Std}} x \frac{Dil_{Smp}}{SampleWt_{Smp}}$$

$$Concentration = Amount / Injection\ Volume$$

Note that SampleWeight and Dilution can be used as any multiplying or dividing factors as the following:

	Sample Weight	**Dilution**
Standard's Amount	Multiplying factor	Dividing factor
Unknown's Amount	Dividing factor	Multiplying factor

The calibration curves for the separate components appear in the *Curves* view and are associated with the *Processing Method* and the results. Make sure to clear the old calibration curve before creating a new one, because the calibration is commulative.