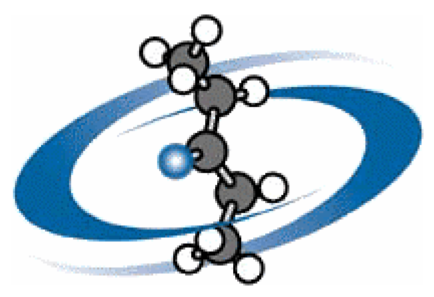

ACD/Method Development Suite
AutoChrom Console

Version 10 for Microsoft Windows

Quick Start Guide

Advanced Chemistry Development, Inc.

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1. Quick Start Guide

1.1 Introduction

ACD/Method Development Suite 10.0 has a number of new features that are not present in version 9. The most striking new feature is the Project Management Interface. This contains the complete workflow and project management support for the experimental optimization portion of method development. This should help you to organize your data files and summarize your results. In addition, there are new capabilities around chemometric peak tracking. Those of you with ACD/Method Development Suite for LC/UV have access to automated peak tracking for your LC-UV data. Those with ACD/Method Development Suite for LC/MS have access to both UV chemometric tools and MS tools.

This Quick Start Guide will teach you how to create a project, build a strategy, import raw data, apply the UV Peak Matching tool to process all of your data at once, select the best run, find optimum conditions, and complete your project.

1.2 Creating a Project

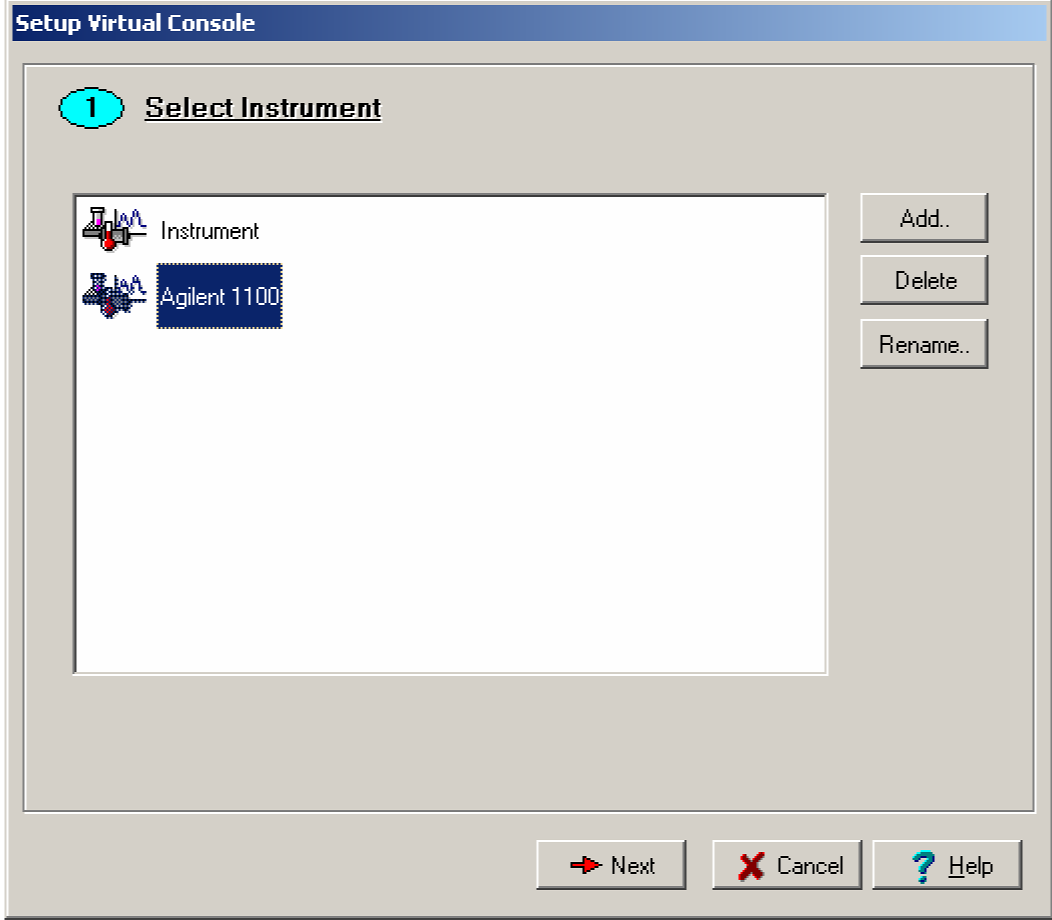
In this section we will create a project and add all conditions that we may or may not use right away. We will be working with the same project throughout the whole document.

1. Start AutoChrom.exe. By default it opens the Task window with several subwindows: Components, Waves, Experiments, and Assistant.

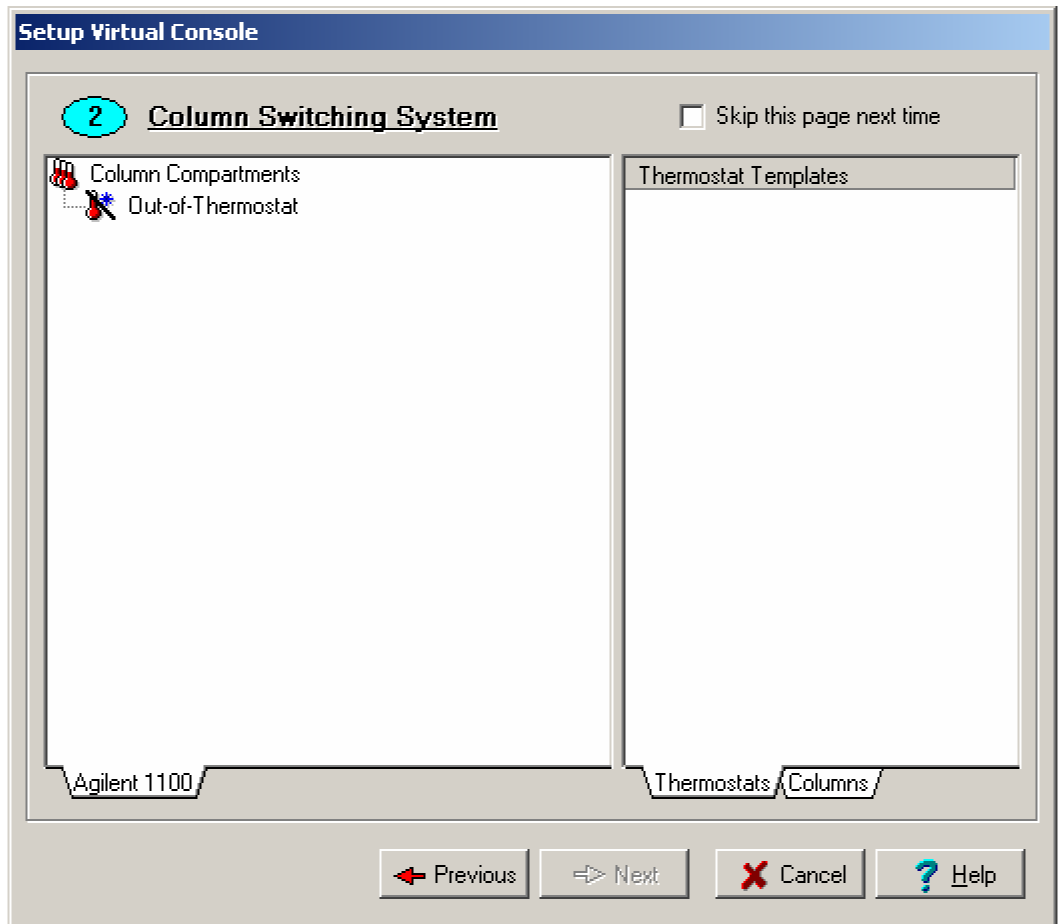
   > **Note** Clicking the tabs on the upper right subwindow will allow you to view some additional information. The **Assistant** tab is a Wizard that will help you to create and maintain the project.

2. Click **Create** in the **Assistant** tab.
3. In the **Open Workspace** dialog box, specify the name and location of the *.awx file and click **Save**.
4. A **Console Selection** window pops up, where you can specify what type of Instrument Console to use. If you have Agilent ChemStation installed, you may see Virtual and Agilent ChemStation options. For this example, choose **Virtual Console** and click **OK**.

5. The **Setup Virtual Console** dialog box allows you to specify your virtual instrument. Click **Add**, in the **Add New Instrument** dialog box, type the name **Agilent 1100** and click **OK**. This instrument will be added to this window and can be used for other projects.

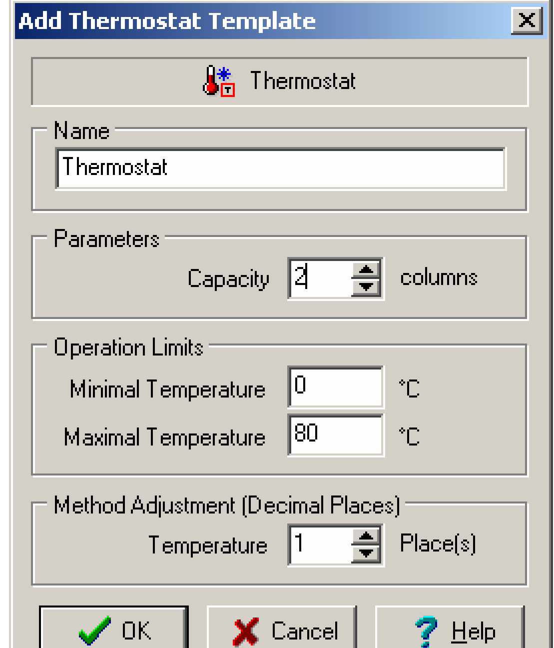


6. With this instrument selected, click **Next**.

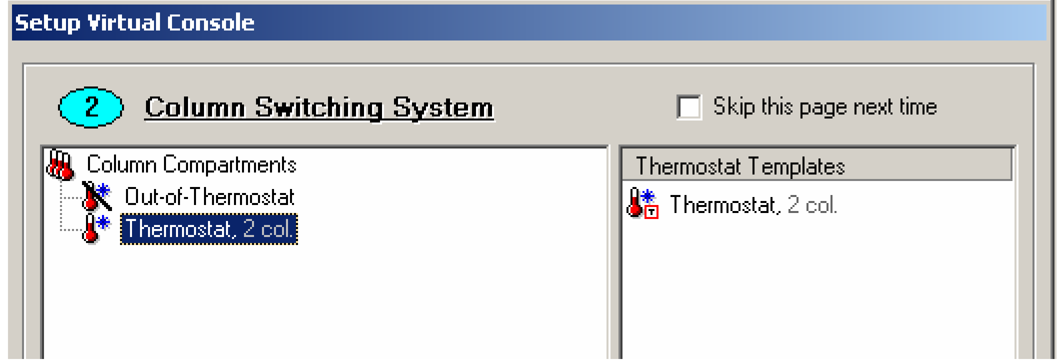


Note While editing the **Setup Virtual Console**, you will observe two subwindows and a few tabs on the bottom. Each item (thermostats, columns, etc.) on the right can be highlighted and dragged to the left. Some of these items are predefined and listed for you. If not, right-click and choose **Add Standard**.

7. On the **Thermostat** tab, right-click and choose **Add**.



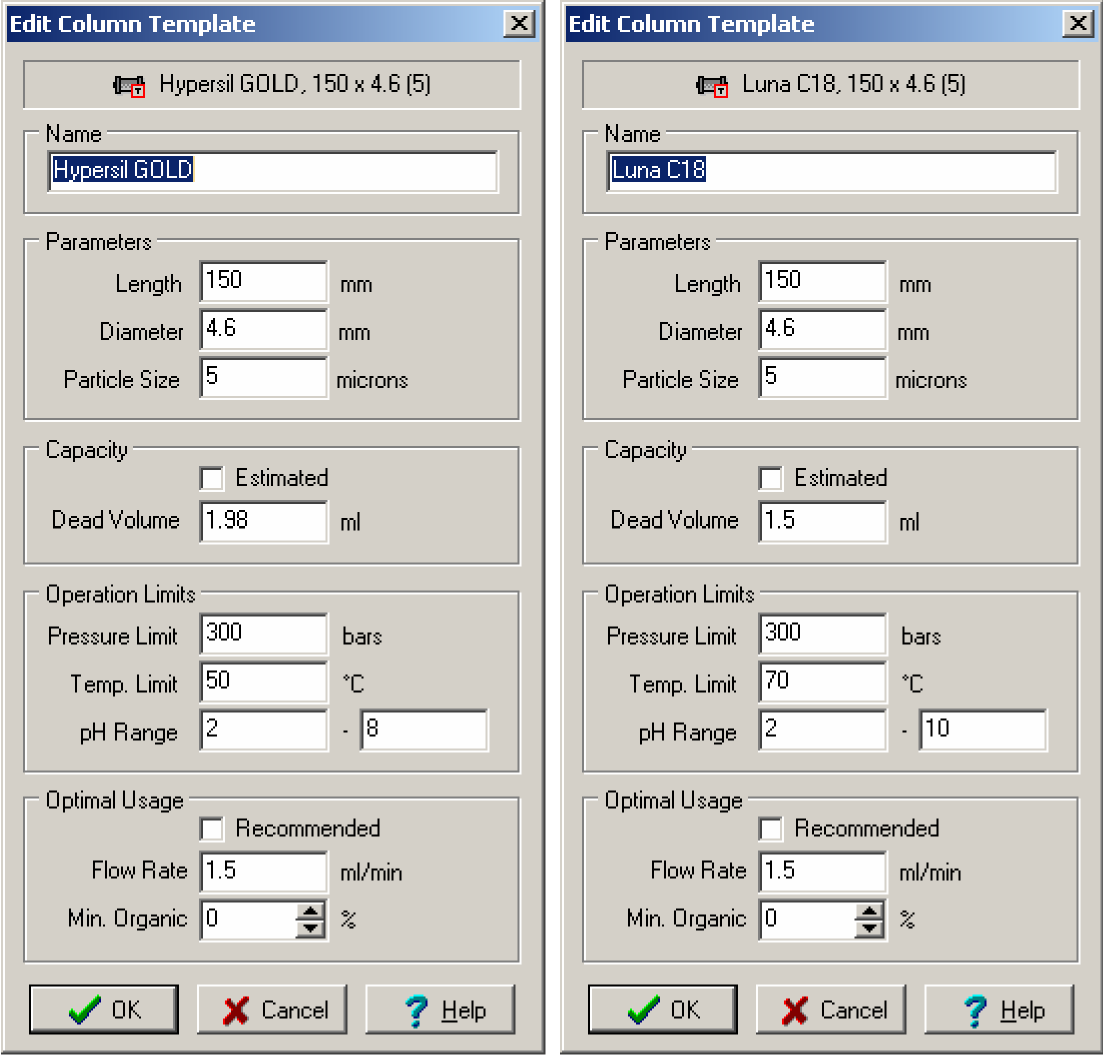
8. Specify the name, capacity, and temperature and click **OK**. It will place a new object to the right.
9. Select this object and drag it to the left.



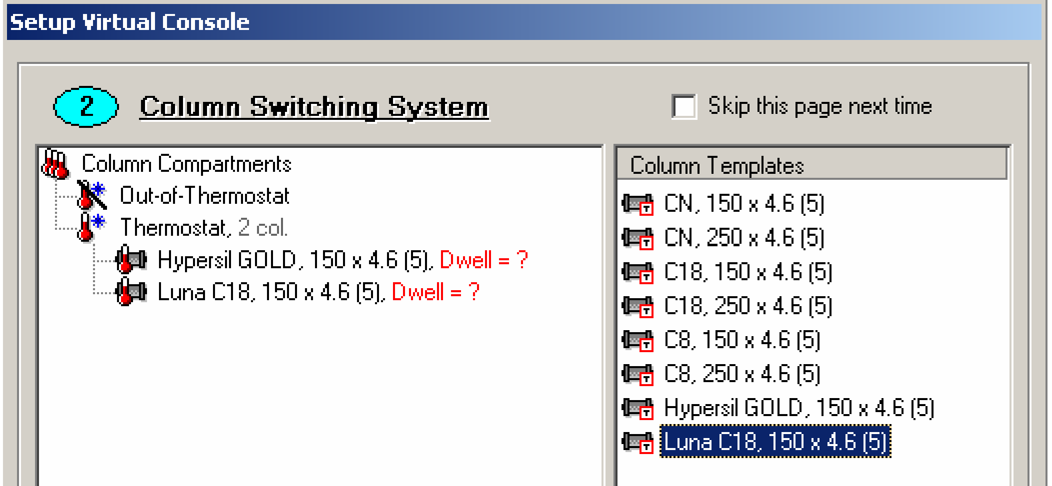
> **Note** You will need a thermostat if you decide to do Temperature Optimization; otherwise you can add columns straight to the left without adding thermostat.

10. Switch to the **Columns** tab.

11. You will see a short list of predefined columns. Right-click on the right subwindow and add two new columns with the following parameters:

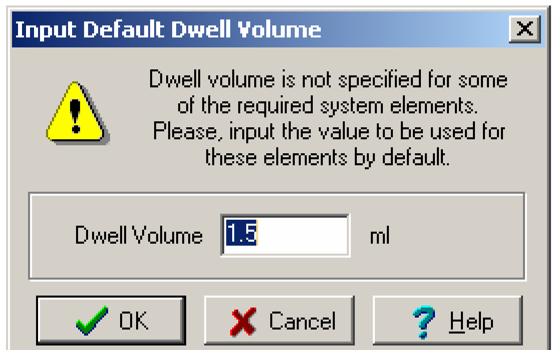


12. Select them one by one and drag to the Thermostat line on the left.

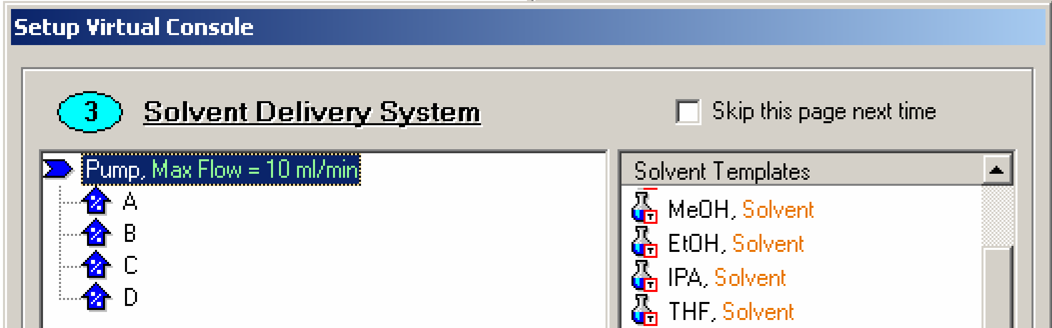


> **Note** Highlight an object on the left and right-click. You will be able to edit, delete, or move it. You cannot drag it back to the right; removing from the active box is done by pressing DELETE.

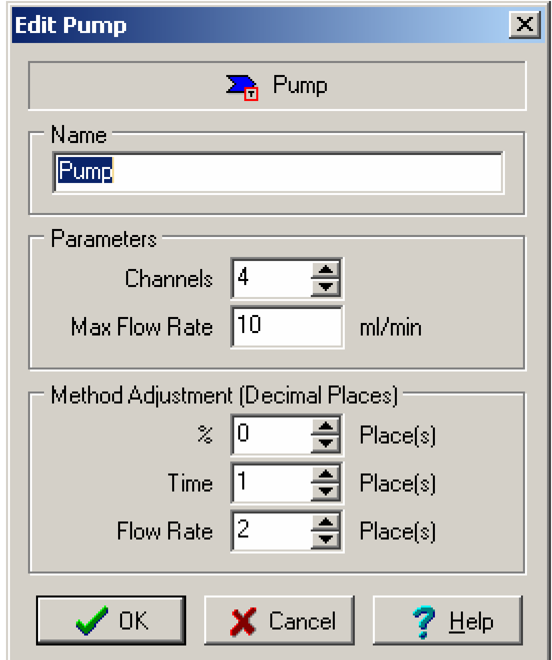
13. Click **Next**.
14. You will be asked for the **Dwell Volume** value if it was not previously submitted. Type in the known value or accept the default one and click **OK**.



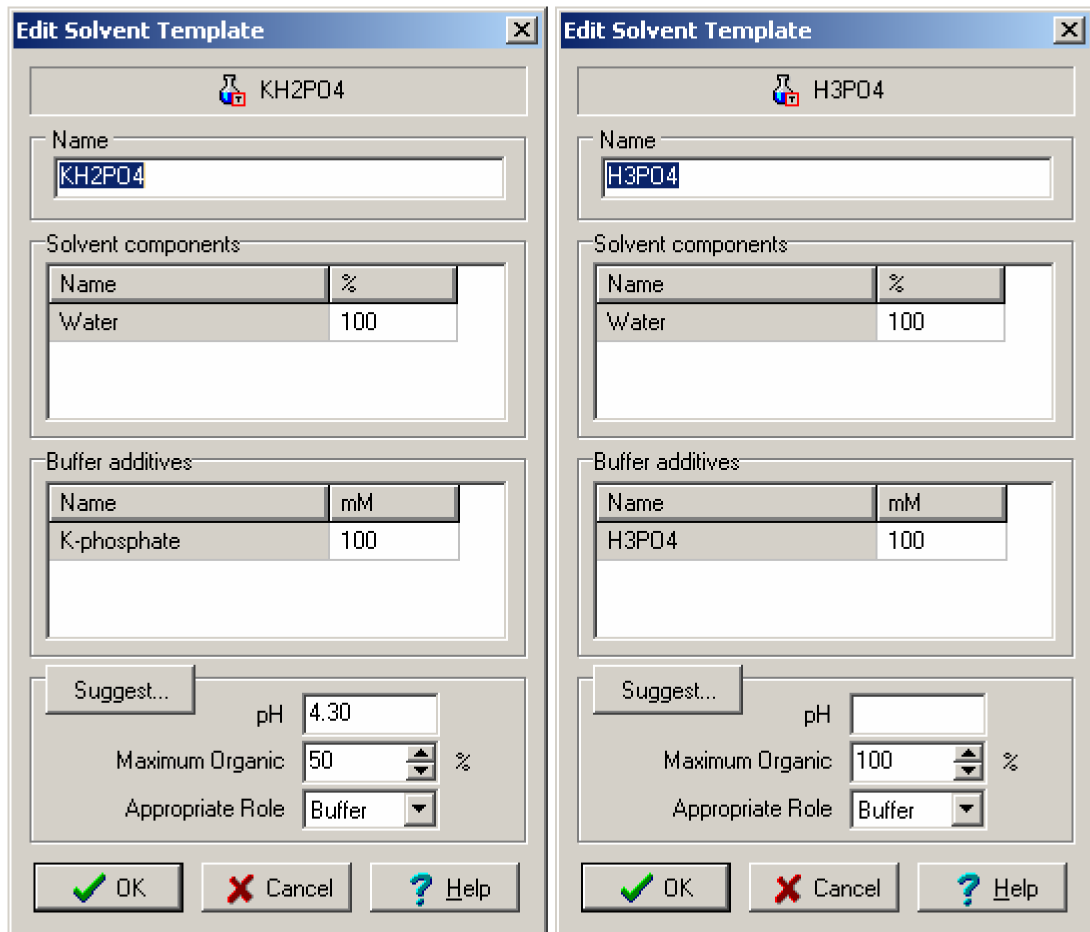
15. You are now ready to submit information about the solvent system. In the left subwindow double-click the Pump line and check the settings for Channels, Max Flow Rate, etc.



16. Make sure the Pump has 4 channels. Click **OK** to close the **Edit Pump** dialog box.

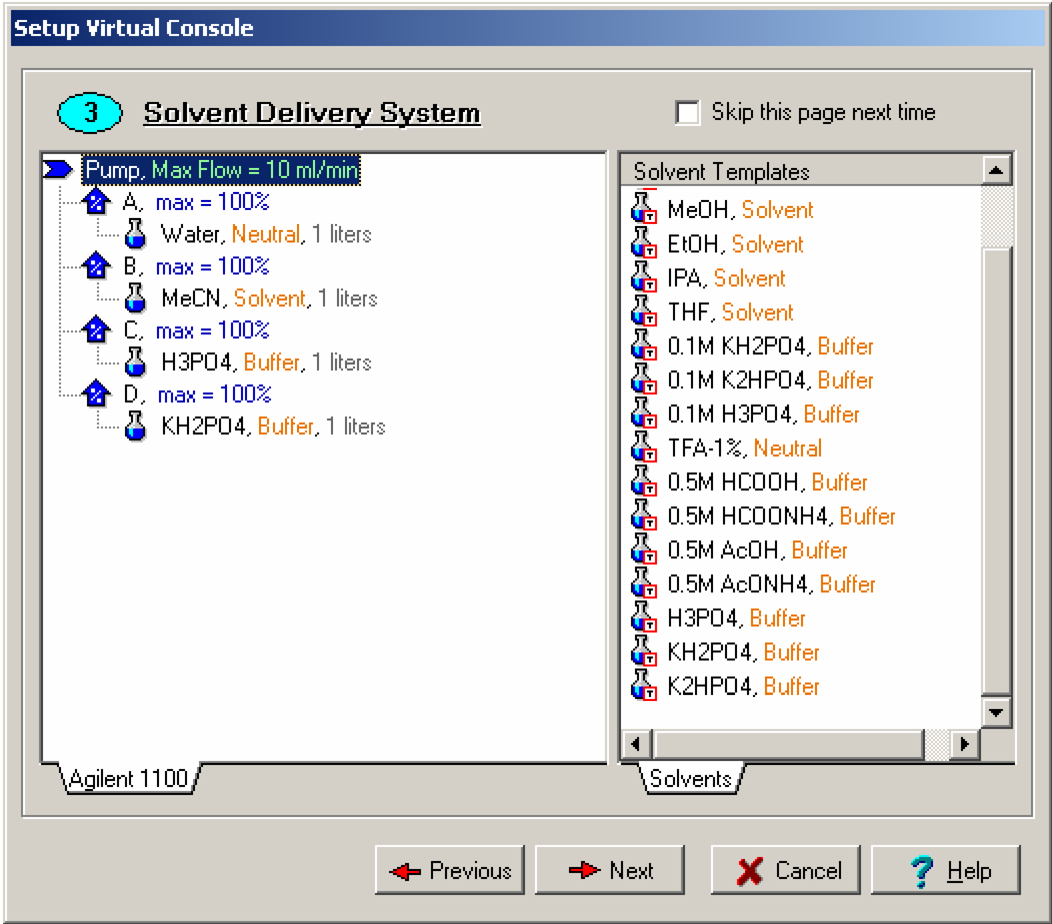


17.Right click on the right subwindow and add two new columns.

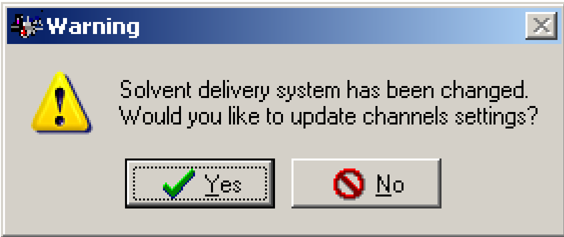


18.Bring the solvents to the left as it shown below.

> **Note** As you may have noticed, we put buffers in different channels. It helps us to mix them if needed and experiment with different pH.

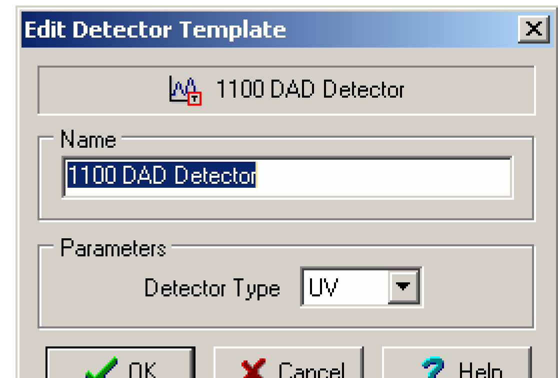


19. Click **Next**.
20. You will see a warning about updating channels settings.

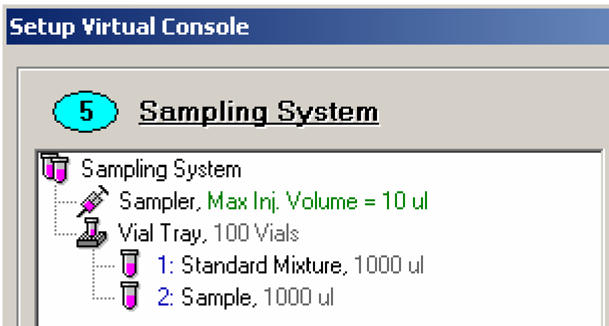


21. Click **Yes**.
22. In the **Edit Channels Settings** dialog box, click **Suggest** and accept these settings.
23. Click **OK** twice.
24. You are now viewing the **Data Acquisition System** window, where you can say what detectors will be utilized and how they are connected: parallel or sequential connection.

25. On the left add a new detector with the following parameters:



26. Drag this detector to the left.
27. Click **Next** to move to **Sampling System**.
28. In the same manner add two samples on the right and drag them over to the left to get the following:



> **Note** Vial is a holder that may hold more than 1 sample.

29. When the samples are complete, click **Finish**. It takes a few seconds to create a project.
30. With Windows Explorer, navigate to the directory where the *.awx file was created. In the same directory there is a folder with the same name that was automatically created, it is now empty. When you populate your project with datasets, the data files are copied and placed into the folder. You can now process them and no changes will be done to your original raw data. You will operate with the copy only.

> **Note** The project is created but we are still observing blank subwindows. Where did all the information go? Switch to the **Workspace** window by clicking the corresponding switching button on the bottom. You will see all the information in the **Instrument** panel.

Exercise:

Close this project and create a new one. Choose the same instrument (Agilent 1100) and click **Next**. Note that all settings are kept with the instrument and you need only click **Next** and **Finish** to create the same project. If you need to make changes, you are allowed to do so, but the instrument will be updated with the latest information.

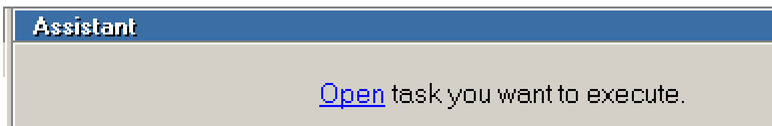
1.3 Sending a Project to Another Person

1. If you are sending a project (i.e., via email) and don't want to send related data sets, you can send simply the *.awx file. When the receiver saves the file, they will not be able to open it until the data folder is present. A folder should be created manually and have the same name as the project and be located in the same directory. This will allow the user to open the *.awx file.
2. If you received an *.awx file and a few data sets, the process is similar to Step 1. Save the *.awx file, create an additional folder with the same name, and place all datasets into this newly created folder.

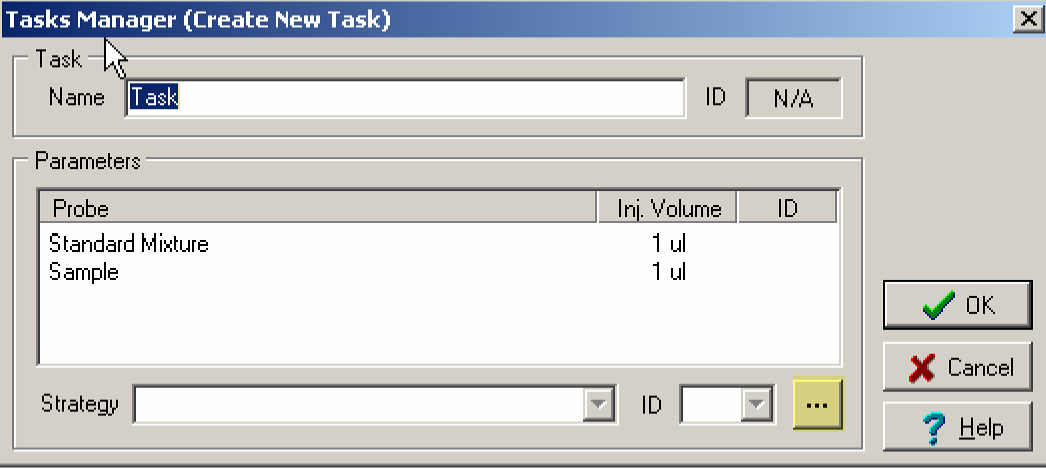
1.4 Building a Strategy

In this section we will build a workflow of what we are planning to select and/or optimize.

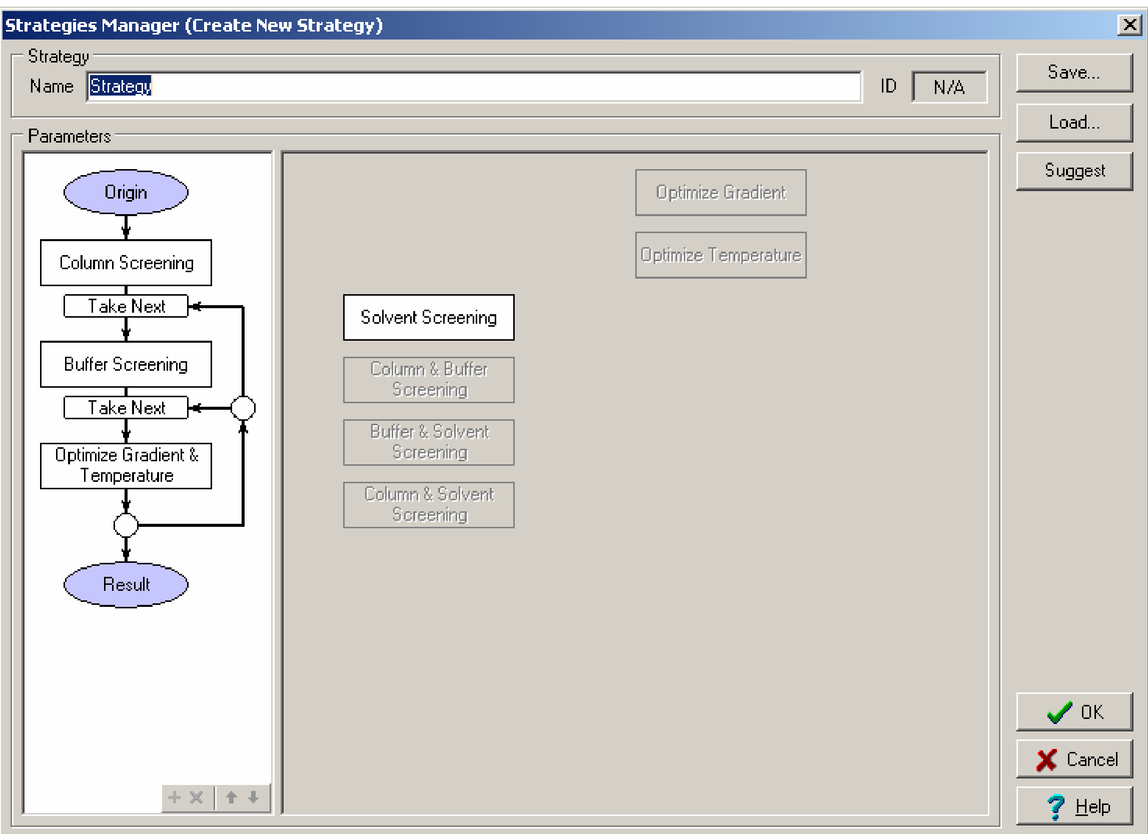
1. In the Assistant tab click Open.



2. The **Tasks Manager** dialog box appears. It will create a task and relate a strategy; the task also needs a name. The **Parameters** (such as **Probe**, **Injection Volume**, and **ID**) are automatically populated from your Sampling System.
3. Click **Browse** next to the **Strategy** and **ID** fields.

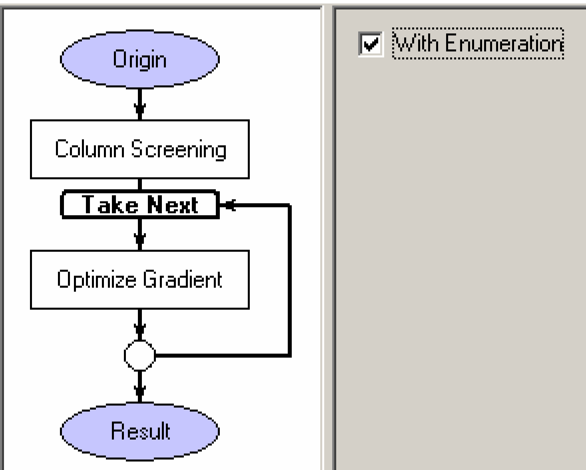


4. The **Strategies Manager** dialog box appears that suggests what to do based on the information you provided while creating a project. Specify the name or accept the default strategy name.

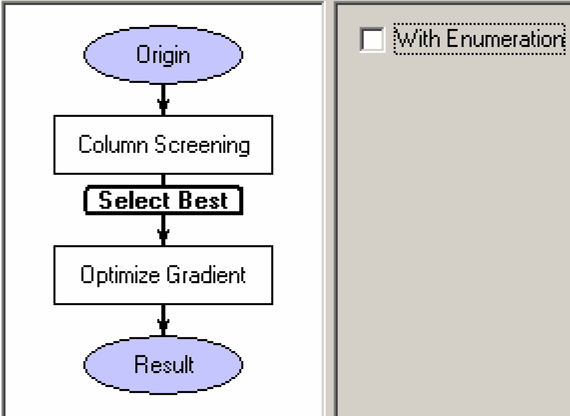


5. We will build our own strategy. Before we do that, we need to remove the objects we don't need.
6. Select the **Buffer Screening** object and click the **red cross** button on the bottom to delete it.
7. In the same manner, delete the **Optimize Gradient & Temperature** object.
8. When deletion is complete, you will see the options on the right that can be added to the strategy.
9. Click on the **Optimize Gradient** object to add it to the strategy.

10. Select the **Take Next** object and clear the **With Enumeration** check box on the right. You will see that the **Take Next** object switches to the **Select Best** object. You can switch it back by selecting this check box.

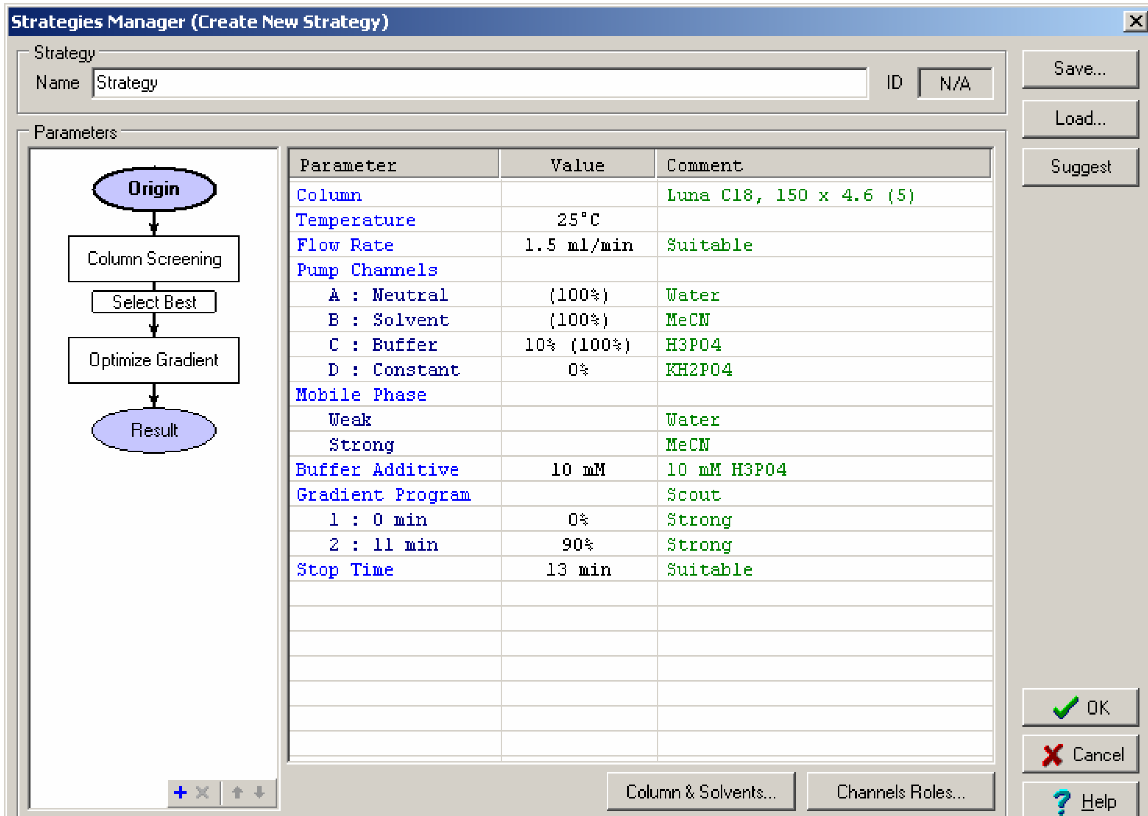


11. As a final strategy you should see this:

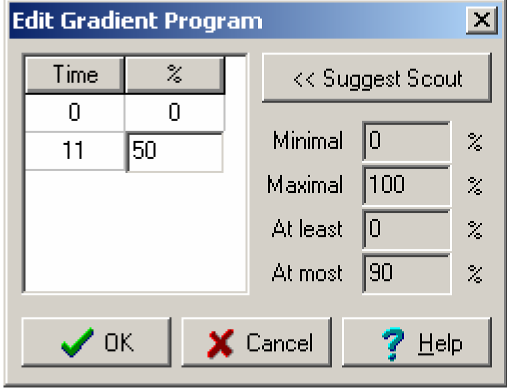


> **Note** Each big object in the strategy is called a wave. So, we built a strategy with two waves: one for Column Screening and one for Optimization.

12.Select the **Origin** object to see more options on the right panel.



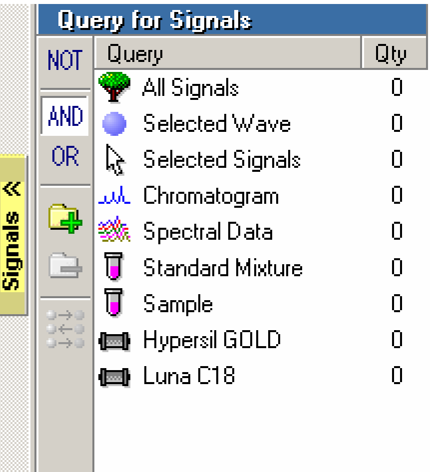
13.Double click the **Gradient Program** line and change the program to the following:



14. Click **OK** three times to accept this strategy and close the **Tasks Manager** window.

> **Note** You can go back to your strategy from **Task Manager** by clicking **Browse** and Rename, Save, or Remove it. To edit the existing strategy, from the **Task** menu, choose **Update Strategy**. Or switch to the **Strategy** tab (i.e., the upper right subwindow) and right-click inside this tab; choose **Update**.

15. Note that as soon as the task and strategy are created, the **Query for Signals** panel is populated.



> **Note** If you do not see this subwindow, click the **Signals** panel on the left that expands and hides two additional subwindows.

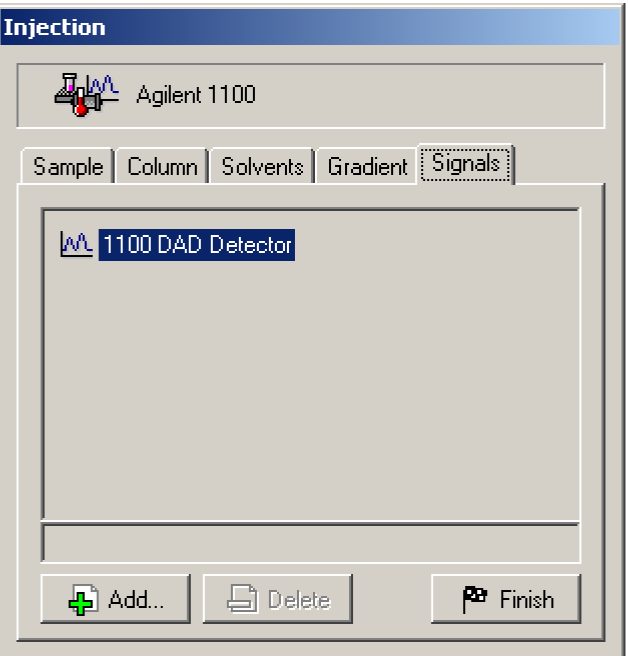
1.5 Working with Task

1.5.1 Importing Data for Wave 1

1. Click the **Start** task link on the **Assistant** tab.
2. The Wave subwindow is now populated with the first wave. And the Experiments subwindow shows what data at what conditions we need to bring to the project at this stage.
3. The Assistant shows new options for further actions. One of them is always stop **the task**. Then you may have the **conditions** or **method** link. And at the end, there is a start execution link.

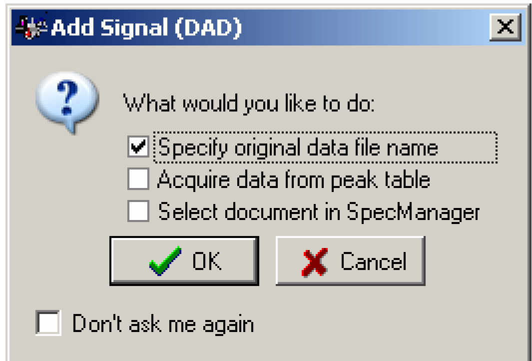
> **Note** If you click one of the conditions links, you will be able to edit the conditions. To do that, when the link is highlighted, switch to **Method** tab. Inside the **Experiment Method** subwindow, click the button you want to edit (i.e., Temperature, Flow Rate, Gradient, Stop Time) and a new pop up window appears where you can make your changes. Or, right-click inside this subwindow and choose one of the **Edit** commands.

4. Click start execution.
5. Click connect **to the instrument** link, even for the virtual instrument. An **Injection** dialog box appears that allows you to bring in your datasets.

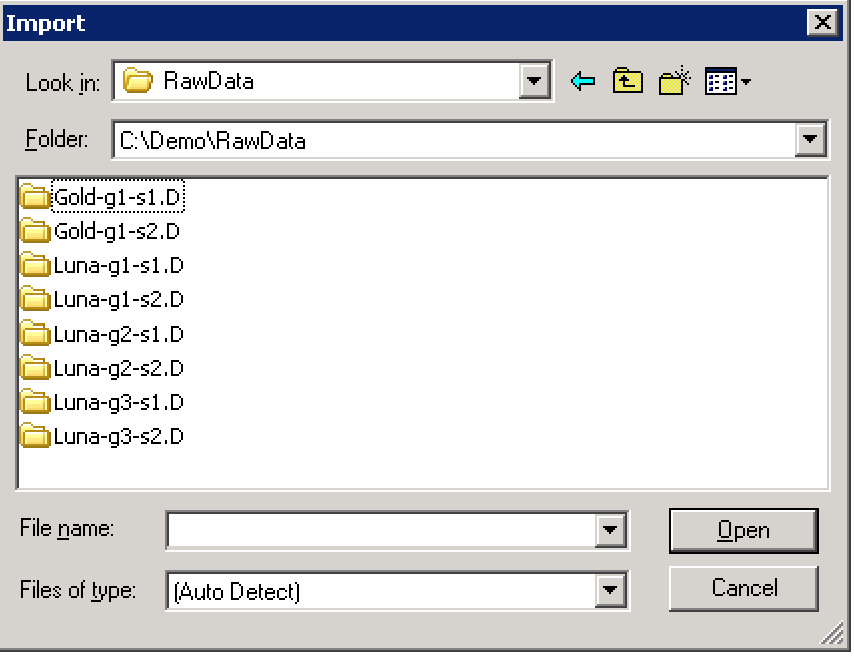


6. On the **Signals** tab, select the detector and click **Add**. If you have only one detector, it will be automatically highlighted.

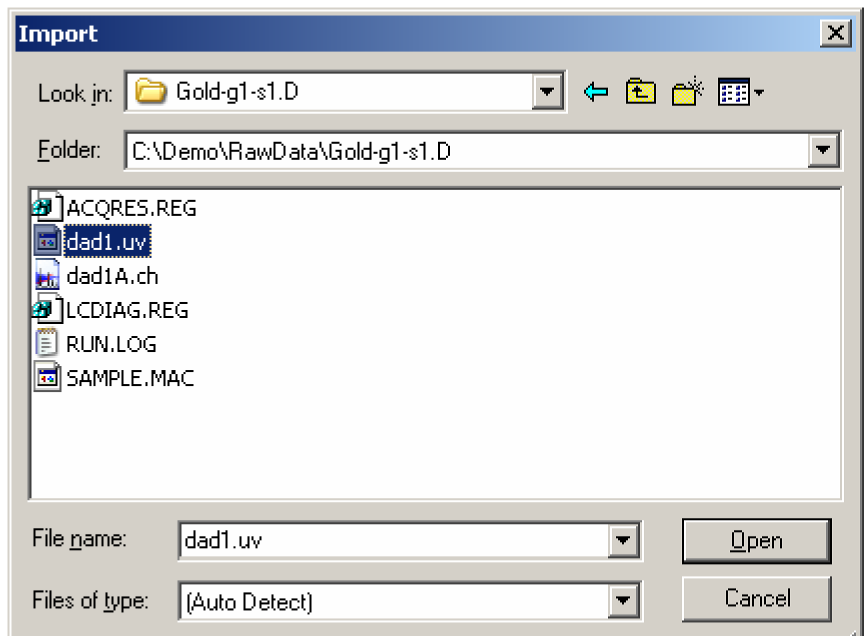
7. You will see 3 options for bringing data in:



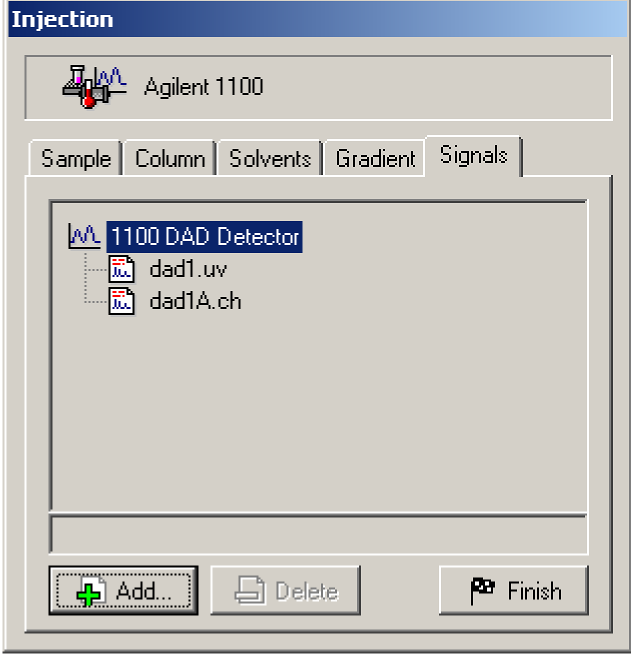
8. You can select the first check box and navigate to the data set in its original location. An alternative is to copy the data from the peak table and paste it when selecting the second choice. While choosing the third option, the program switches you to the Processor window, where you have open files, select the required file and click **OK** on the bottom to return to AutoChrom Console, Task window.
9. For this example, select the **Specify original data file name** check box and click **OK**.
10. In the **Import** dialog box, navigate to the folder with raw data and choose the Gold_g1-s1.D subfolder and click **Open**.



11.When you are inside this subfolder, navigate to the dad1.uv file and click **Open**.



12.As a result, data will be added to the Injection window.

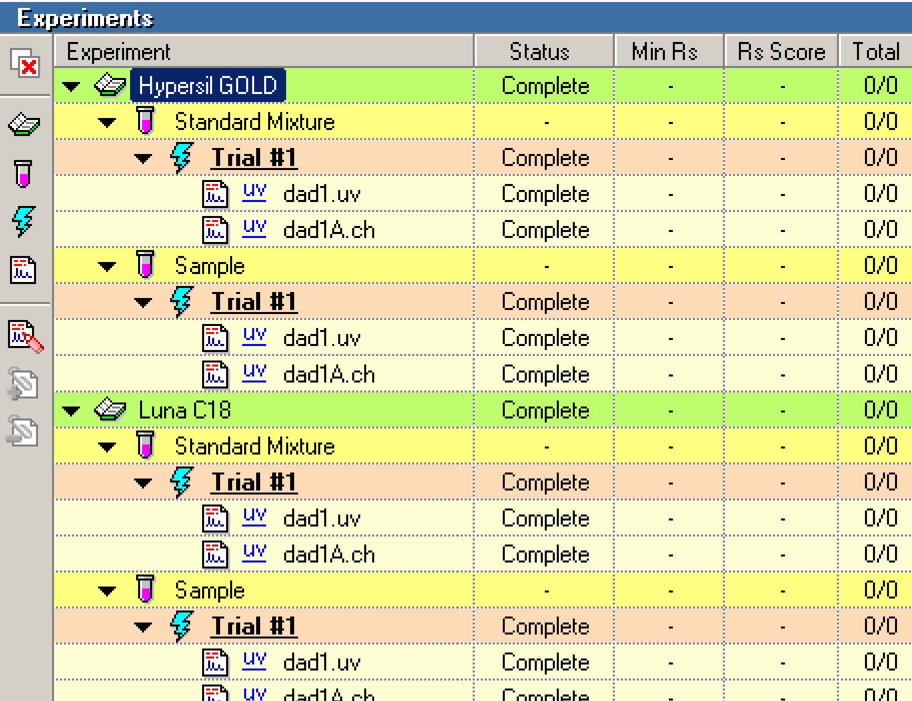


13.Click **Finish** and this dataset will be imported to the project. The same window will pop up again asking you to bring more data.

14.Repeat steps 6-13 and keeping the exact order, bring data from the following subfolders: Gold-g1-s2.D, Luna-g1-s1.D, and Luna-g1-s2.D.

> **Note** When you will create your own project, you will know what data to import for each injection.

15.Let’s look at the imported data:



Experiments

Experiment	Status	Min Rs	Rs Score	Total
Hypersil GOLD	Complete	-	-	0/0
Standard Mixture	-	-	-	0/0
Trial #1	Complete	-	-	0/0
UV dad1.uv	Complete	-	-	0/0
UV dad1A.ch	Complete	-	-	0/0
Sample	-	-	-	0/0
Trial #1	Complete	-	-	0/0
UV dad1.uv	Complete	-	-	0/0
UV dad1A.ch	Complete	-	-	0/0
Luna C18	Complete	-	-	0/0
Standard Mixture	-	-	-	0/0
Trial #1	Complete	-	-	0/0
UV dad1.uv	Complete	-	-	0/0
UV dad1A.ch	Complete	-	-	0/0
Sample	-	-	-	0/0
Trial #1	Complete	-	-	0/0
UV dad1.uv	Complete	-	-	0/0
UV dad1A.ch	Complete	-	-	0/0

16.If your file was previously processed, the **Experiments** window will show all the peak names and retention times. The **Components** table will also be populated where you can add and edit the structure and perform many other operations using the shortcut menus.

Important When you process your data in the Processor window, make sure the peak names in the table of peaks are not specified as unknown. The Unknown name is given by default to all labeled peaks when you perform Peak Picking. You will need to rename them to something else in order to see the peaks and associated retention time values in the Task window

1.5.2 Processing Data in Wave 1

1.5.2.1 Editing Dataset in SpecManager

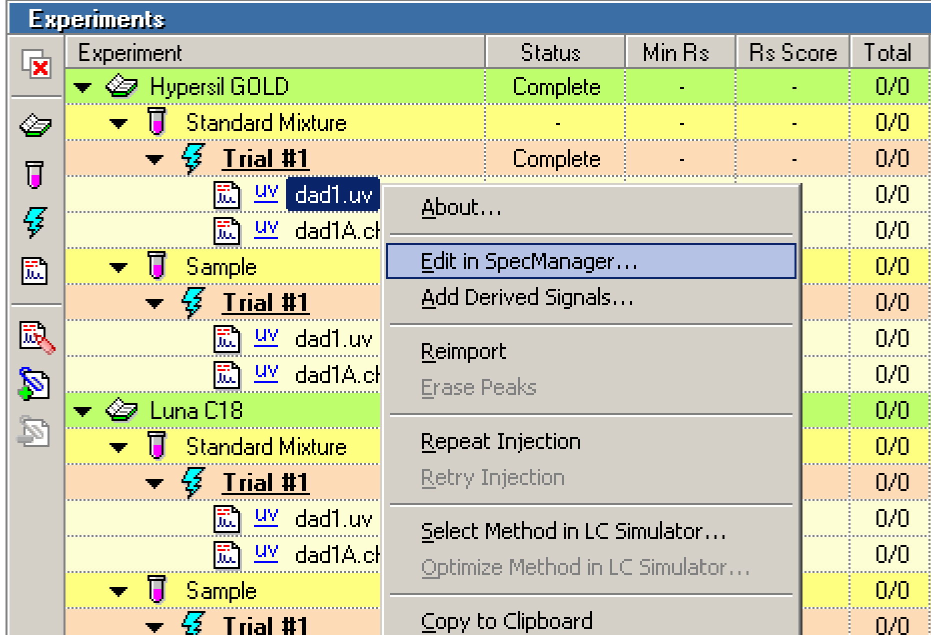
Before accepting injections in the **Assistant** tab, it is recommended to process the data. We are going to find and label the main peaks and match the peaks between runs by comparing the UV spectra for each peak.

There are two ways to process data for the project: in SpecManager or inside the Task window.

First you will learn how to bring data into SpecManager, process it, and bring back to the Task window. But our main focus will be on grouping data and applying the UV Peak Matching tool.

1. In the Experiments table, select a **dad1.uv** signal as shown below, and right-click.

2. From the pop-up menu, choose **Edit in SpecManager**.



3. The program opens a special mode of ACD/SpecManager and the highlighted dataset is imported.

   Important This mode of ACD/SpecManager interacts with the AutoChrom Console and has **OK** and **Cancel** buttons on the bottom. If your processing is complete and you would like to accept it and bring the result back to the Task window, click **OK**. If you have completed all of your investigation and do not want to accept the result, click **Cancel**. You must use these two buttons to complete or abort the analysis in order to close SpecManager.

4. In the Processor window of SpecManager you are now viewing the LC-UV dataset. You can enter Peak Picking mode and label the peaks manually or you can click 2D Processing 2D-Processing and, using the options, find the peaks of interest automatically. Note, the same options are used by the UV Peak Matching tool in AutoChrom Console.

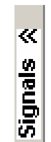
5. We recommend finding the appropriate settings here and recording them. You will generally use these same settings for each use of the UV Peak Matching tool. For this example, you can skip this step.

6. Leave the 2D Processing mode by clicking **Cancel Operation** .

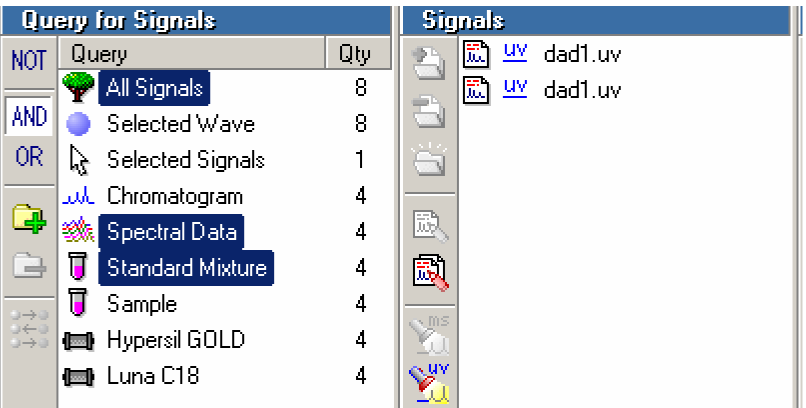
7. Click **Cancel** on the bottom to switch to the Task window.

   Important If you have more than one subsample, you may need to find settings separately for each subsample.

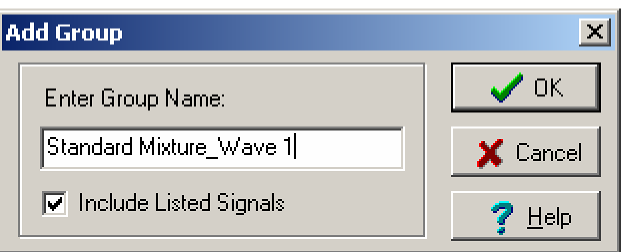
1.5.2.2 Applying the UV Peak Matching Tool

1. Click **Signals** 

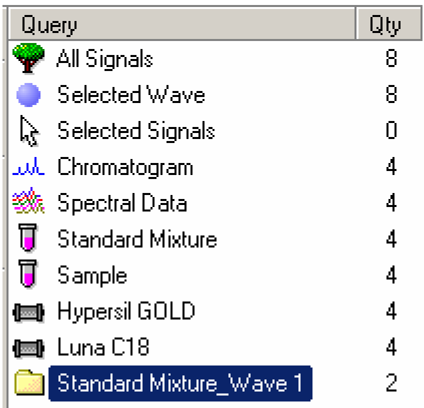
to expand the **Query for Signals** and **Signals** subwindows.
2. In this example we have two subsamples, therefore we will process them separately. In the **Query** section, select All Signals and, while holding CTRL, select Spectral Data and your first subsample—Standard Mixture.



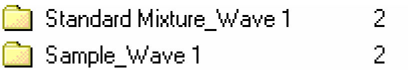
3. We are going to place data of different subsamples into two different groups. With the three lines selected, click **Add Group** .
4. In the **Add Group** dialog box, specify the name, select the check box as shown below and click **OK**.



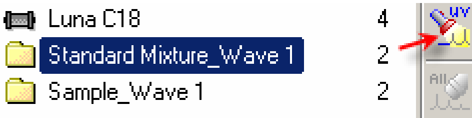
5. A new group is created.



6. Holding CTRL, select the All Signals, Spectral Data, and Sample lines and create another group. Name it **Sample_Wave 1**. You should have two groups now and we are ready to apply the UV Peak Matching tool separately to each group.

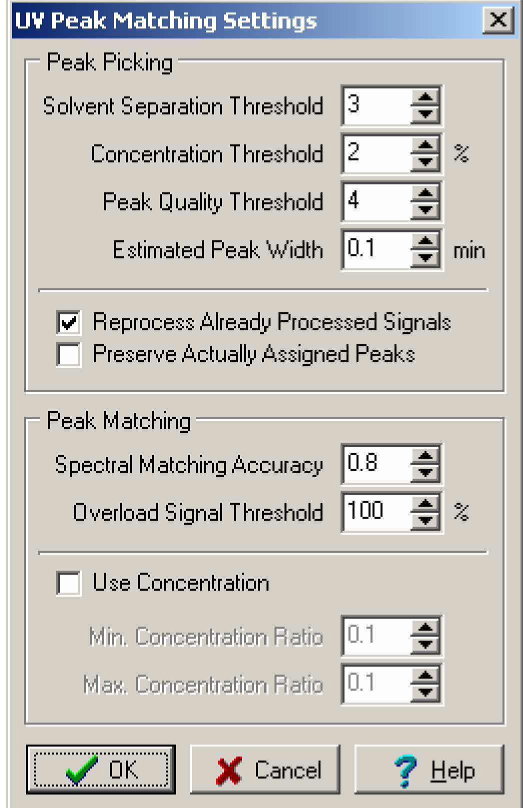


7. Select the first group, Standard Mixture_Wave1, and click **Apply UV Peak Matching.**

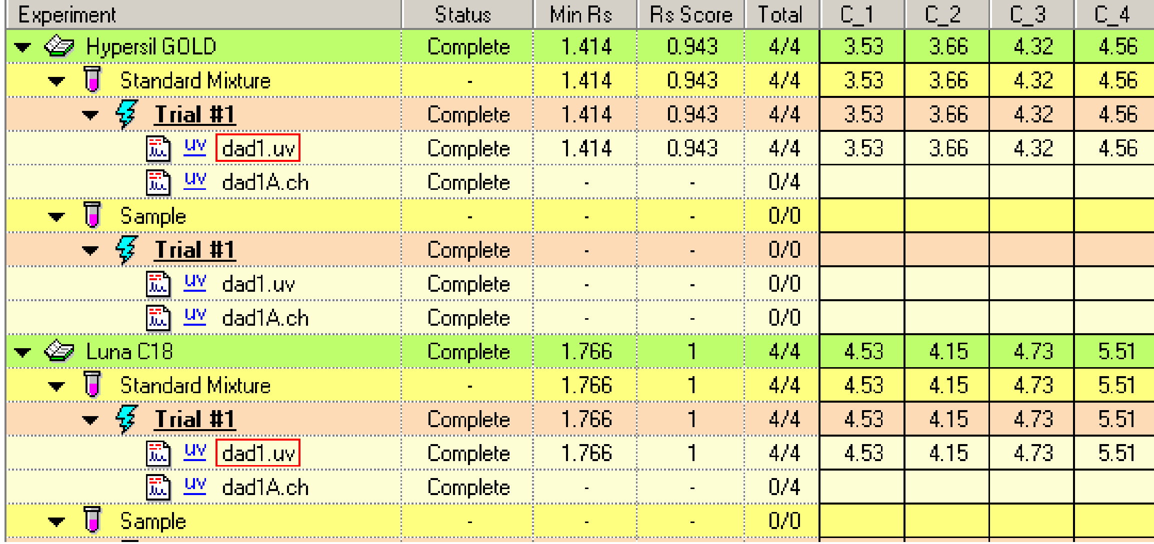


> **Note** If you work with non-hyphenated data, some buttons on the side toolbars will be disabled. For instance, the important tools such as MS-Map and UV-Map are enabled only for signals from hyphenated detectors.

8. In the **UV Peak Matching Settings** dialog box, specify the settings as shown below and click **OK**.



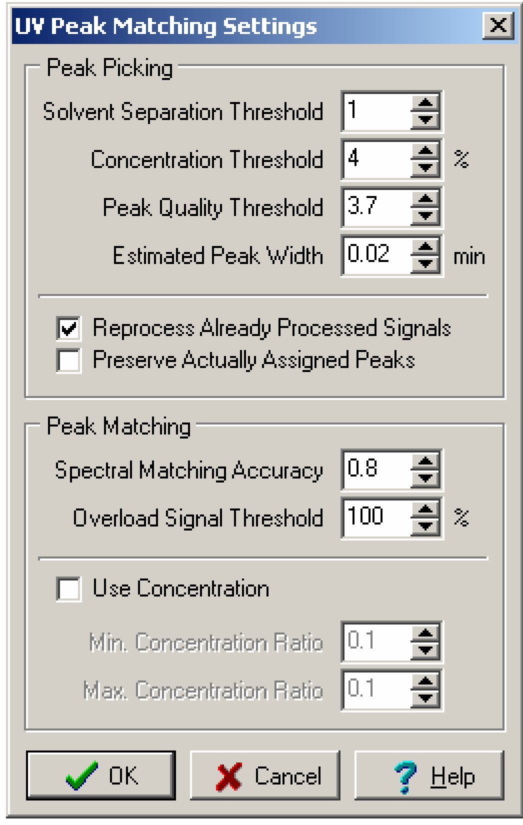
9. The algorithm starts running. It will peak the main peaks, compare the UV spectra for each peak, and reconcile the peaks between datasets. At the same time the found components are named C_1, C_2, and so on.



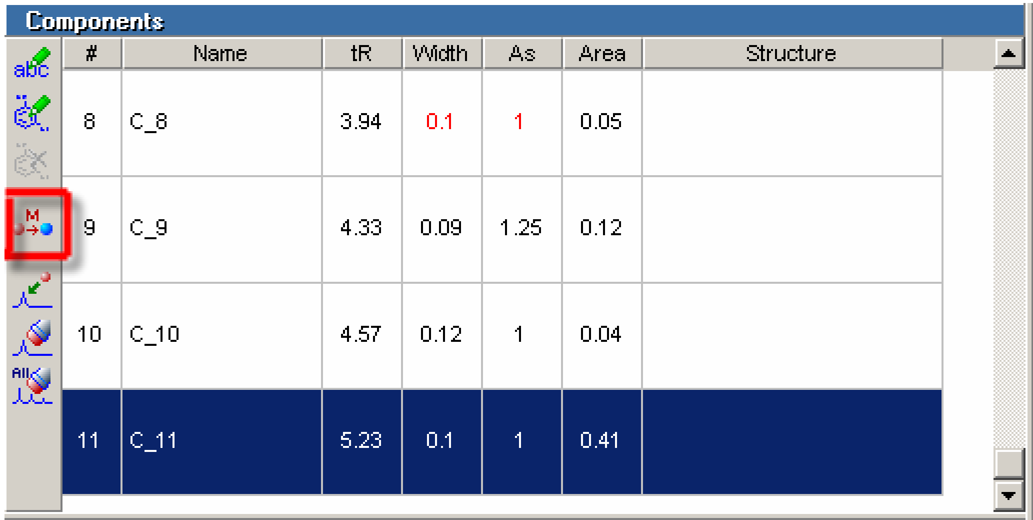
Experiment	Status	Min Rs	Rs Score	Total	C_1	C_2	C_3	C_4
Hypersil GOLD	Complete	1.414	0.943	4/4	3.53	3.66	4.32	4.56
Standard Mixture	-	1.414	0.943	4/4	3.53	3.66	4.32	4.56
Trial #1	Complete	1.414	0.943	4/4	3.53	3.66	4.32	4.56
UV dad1.uv	Complete	1.414	0.943	4/4	3.53	3.66	4.32	4.56
UV dad1A.ch	Complete	-	-	0/4				
Sample	-	-	-	0/0				
Trial #1	Complete	-	-	0/0				
UV dad1.uv	Complete	-	-	0/0				
UV dad1A.ch	Complete	-	-	0/0				
Luna C18	Complete	1.766	1	4/4	4.53	4.15	4.73	5.51
Standard Mixture	-	1.766	1	4/4	4.53	4.15	4.73	5.51
Trial #1	Complete	1.766	1	4/4	4.53	4.15	4.73	5.51
UV dad1.uv	Complete	1.766	1	4/4	4.53	4.15	4.73	5.51
UV dad1A.ch	Complete	-	-	0/4				
Sample	-	-	-	0/0				

10. In the **Query** section, select the Sample_Wave1 group, and click **Apply UV Peak Matching**.

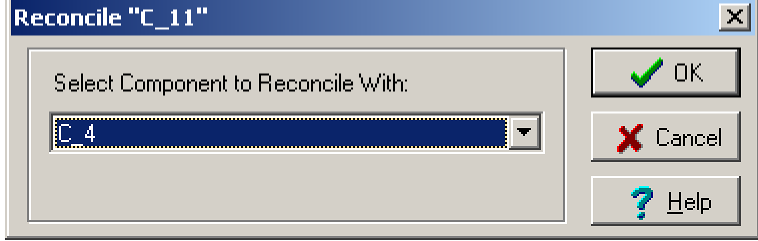
11. For this group, apply the following settings and click **OK**.



12. After the processing is complete, switch to the **Chromatogram** or **Spectrum** tab. Selecting the line or peak in the Experiments table, you will view the composite chromatogram, reconstructed chromatogram or UV spectrum correspondingly.
13. Navigate to the peaks in the C_4 and C_11 columns and note that the UV spectra are similar. We can manually reconcile these columns. In the Components table, select the C_11 component and click **Reconcile Components Manually**.



14. In the **Reconcile** dialog box, in the **Select Component to Reconcile With** list, click **C_4**, and then click **OK**.



15. In the same manner, manually reconcile C_8 with C_1. The Experiments table looks cleaner and we are ready to continue with the project.

C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_9	C_10
3.74	3.66	4.32	4.9	3.03	3.71	3.81	4.33	4.57
3.53	3.66	4.32	4.56					
3.53	3.66	4.32	4.56					
3.53	3.66	4.32	4.56					
3.94			5.23	3.03	3.71	3.81	4.33	4.57
3.94			5.23	3.03	3.71	3.81	4.33	4.57
3.94			5.23	3.03	3.71	3.81	4.33	4.57
4.54	4.15	4.73	5.51	1.13	4.41	4.14	5.07	
4.53	4.15	4.73	5.51					
4.53	4.15	4.73	5.51					
4.53	4.15	4.73	5.51					
4.55			5.51	1.13	4.41	4.14	5.07	
4.55			5.51	1.13	4.41	4.14	5.07	
4.55			5.51	1.13	4.41	4.14	5.07	

Exercise:

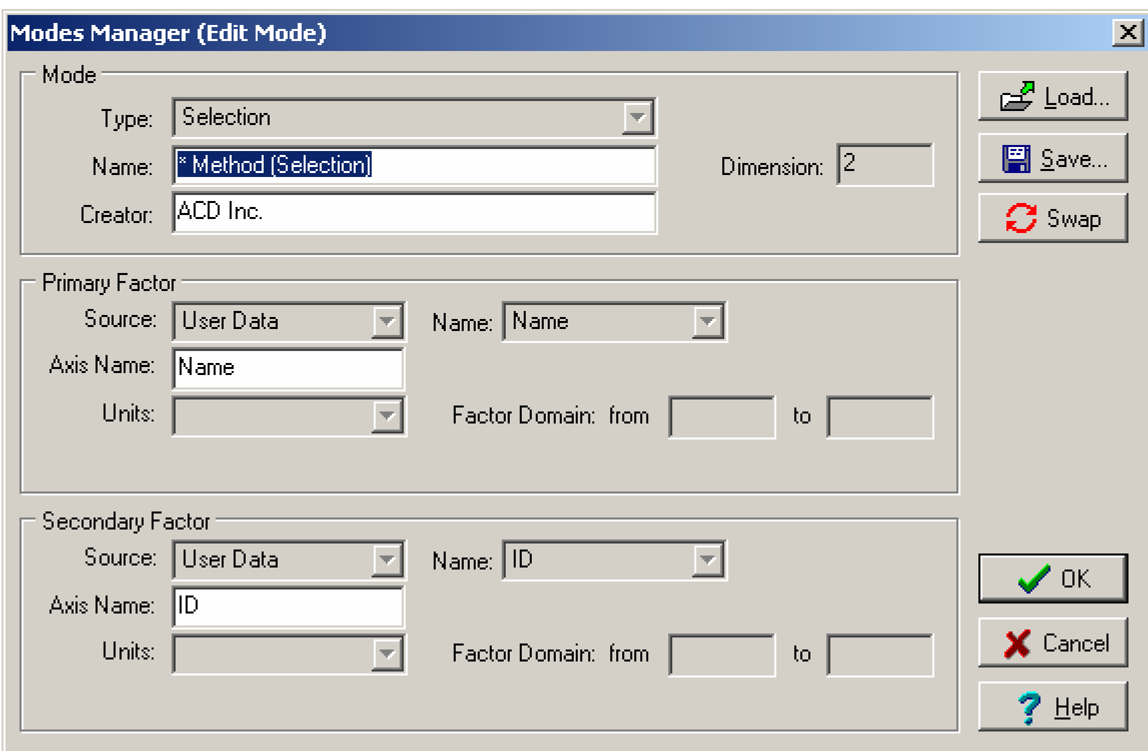
Inside the Components Table, attach a structure of chlorogenic acid to the C_1 line and rename the peak Chlorogenic Acid. For C_2, C_3, and C_4, repeat the same procedure with respect to theobromine, theophylline, and caffeine.

1.6 Selecting the Best Column

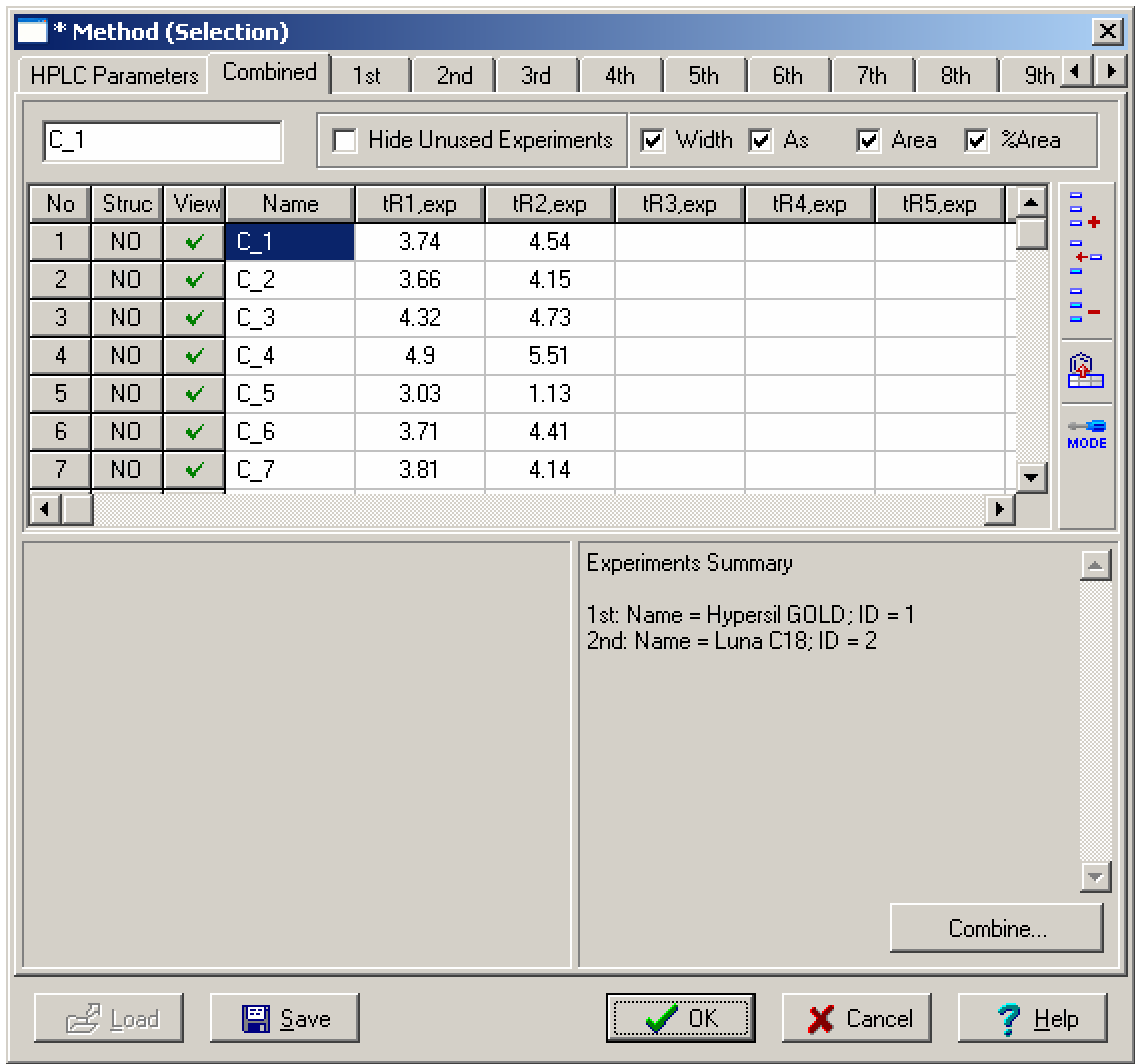
In this section we will transfer data to LC Simulator and select the best column to continue further with optimization.

1. On the **Assistant** tab, choose accept these injections.
2. You see a few new options appear. Select with assistance of ACD/LC Simulator.

3. ACD/LC Simulator starts up. In the dialog box, you can see that the program read the data conditions and picked the Selection mode. Click **OK**.

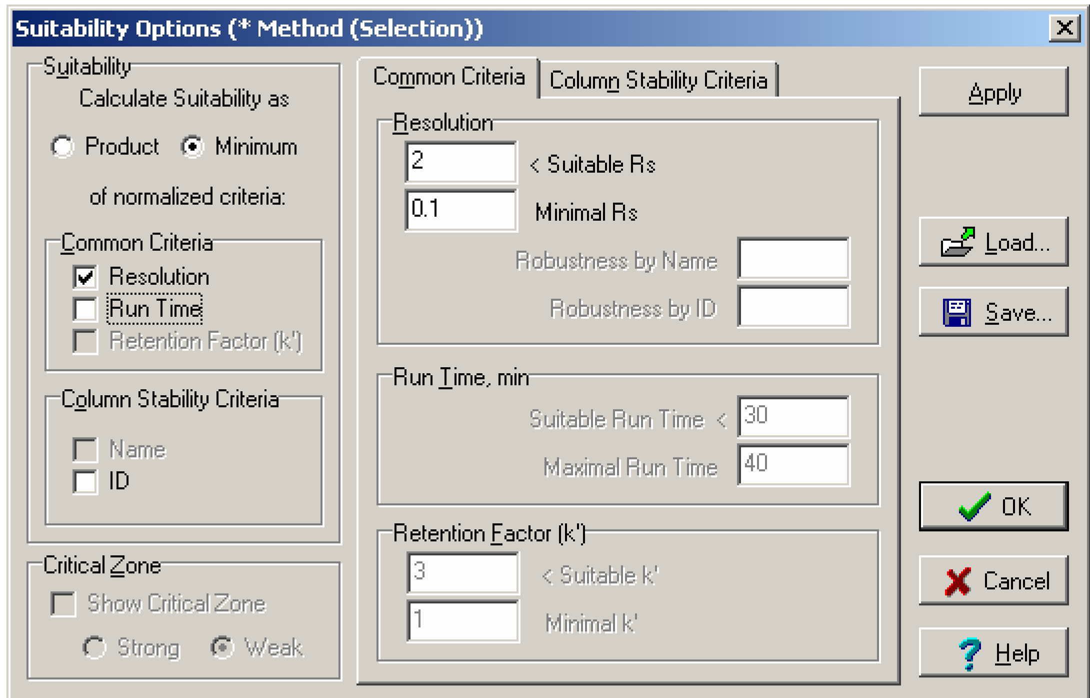


4. All data are combined automatically.



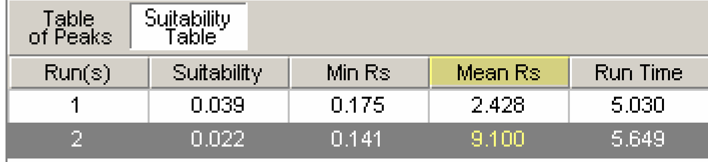
5. Click **OK**.

6. Set the following suitability options:



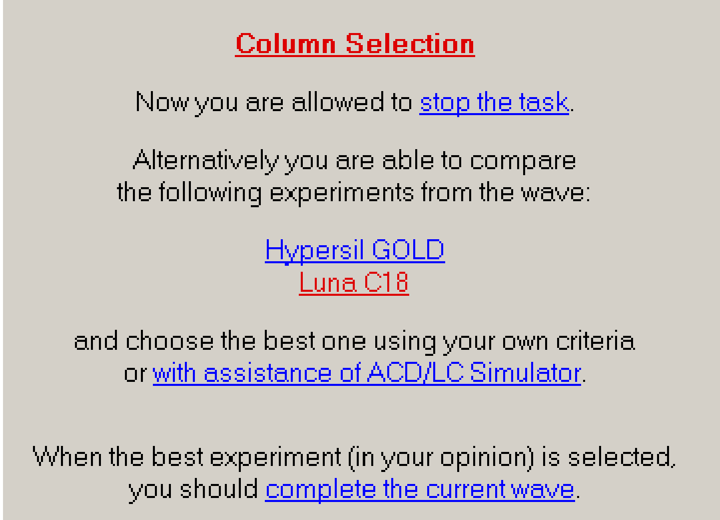
7. Visually, the second run where the Luna C18 column was used looks better.

8. In the Suitability Table you look at the Mean Resolution instead of Suitability as we are interested in the average resolution of all chromatogram peaks to be able to more easily optimize our method. You can see that Luna C18 gives a better result.

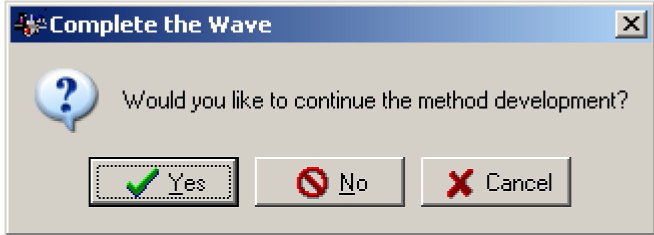


Run(s)	Suitability	Min Rs	Mean Rs	Run Time
1	0.039	0.175	2.428	5.030
2	0.022	0.141	9.100	5.649

9. With this line selected, click **OK** on the bottom of the window and this column will be selected as the best in the **Assistant** tab. The best conditions can be picked manually by selecting it inside the Assistant tab.



10. Click complete the current wave.
11. Click **Yes** to continue method development.



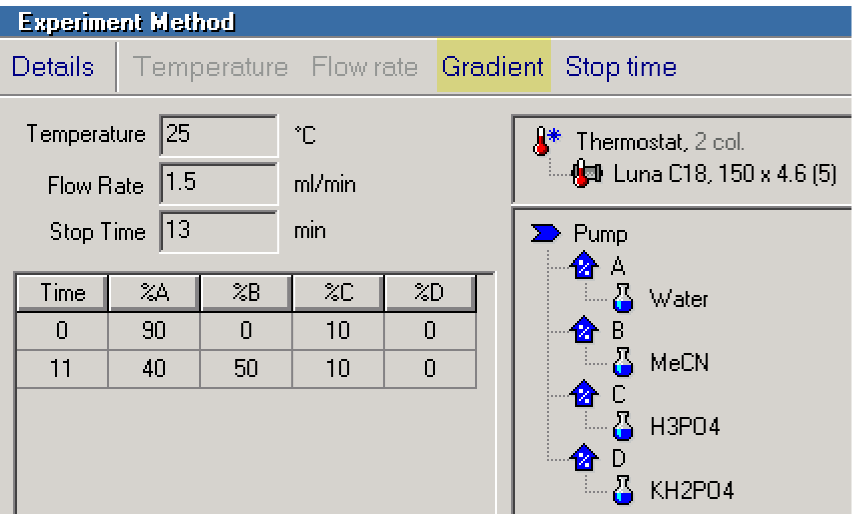
12. The project now proceeds into the next wave and the Waves subwindow shows the best conditions that were selected in the previous wave (Luna C18).

13. Notice that the Experiments table has been changed.

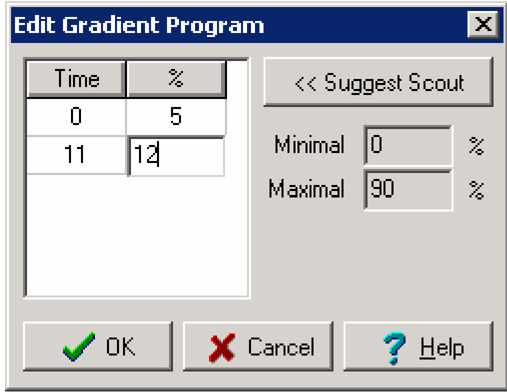
1.7 Importing Data to Wave 2

In this section we will import more data sets with a different gradient program. In the Experiments table, you now see one dataset that came from Wave 1 as the best selected run. There are two more experiments added that show suggested gradient programs to run. As you may have already prepared the datasets with other conditions, the program allows you to change the suggested conditions to what you have.

1. On the **Assistant** tab you can see two suggested gradient programs. Select the first one and switch to the **Method** tab.
2. Click **Gradient**.

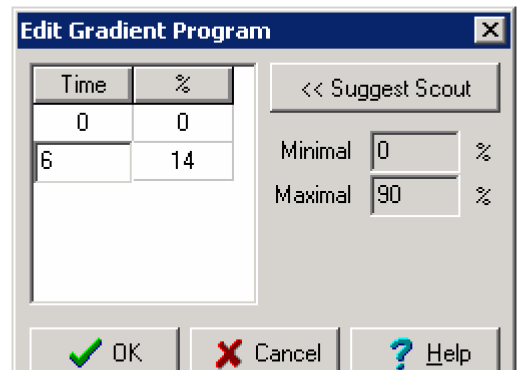


3. In the **Edit Gradient Program** dialog box, change the program to the following:



4. Click **OK**.
5. Go to the **Assistant** tab again and select second condition line. Alternatively, you can just select the next suggested Experiment line and the **Method** tab will show corresponding method.

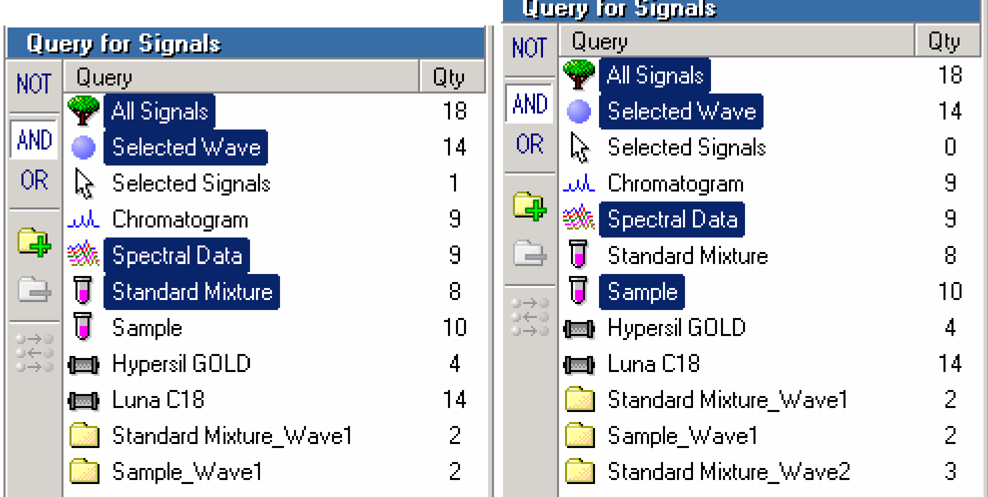
6. On the **Method** tab, click **Gradient** and change the program to the following:



7. You will be prompted to adjust the stop time. Click **Yes** to close this message.
8. Come back to the **Assistant** tab and click start execution.
9. Then click connect and you are ready to import datasets to the Injections window.
10. Bring in the following data files: Luna-g2-s1.D, Luna-g2-s2.D, Luna-g3-s1.D, and Luna-g3-s2.D. You are ready to process new data.

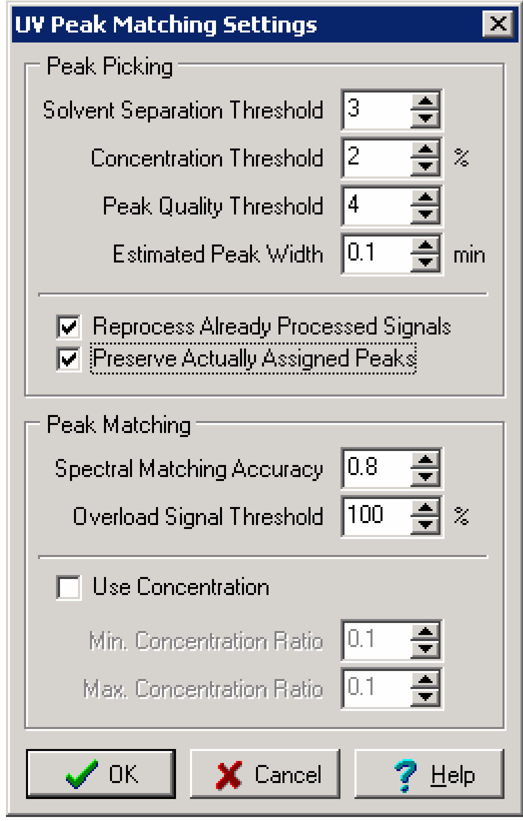
1.8 Processing Data in Wave 2

1. Create two new groups: Standard Mixture_Wave2 and Sample_Wave2. To create these groups, make the following selection.

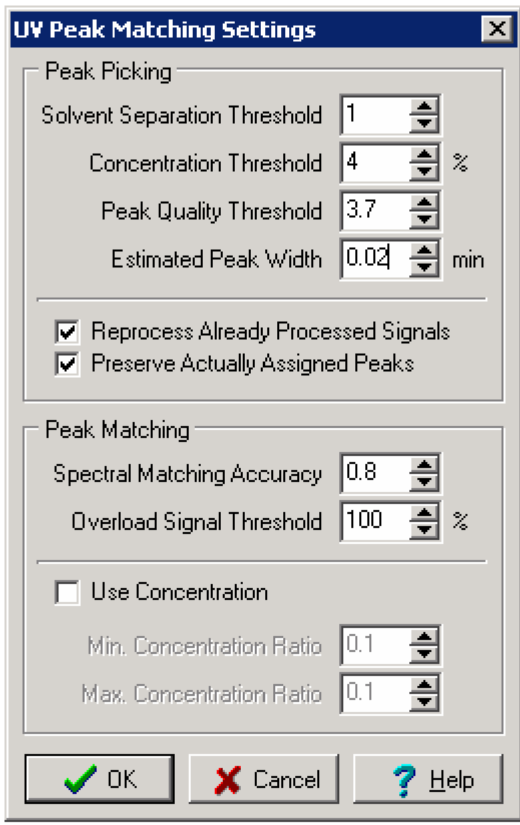


Note The detailed instructions were described in section 1.5.2.2.

2. We will process each group separately. Select the Standard Mixture_Wave2 and click the UV Peak Matching button.
3. Select these settings and click **OK** to start processing. Note that now you have to select the **Preserve Actually Assigned Peaks** check box.



4. Select the Sample_Wave2 group and click **UV Peak Matching**. Select the following settings and click **OK** to start processing.



5. You can see that new peaks are fully reconciled with the peaks in previously processed data sets.

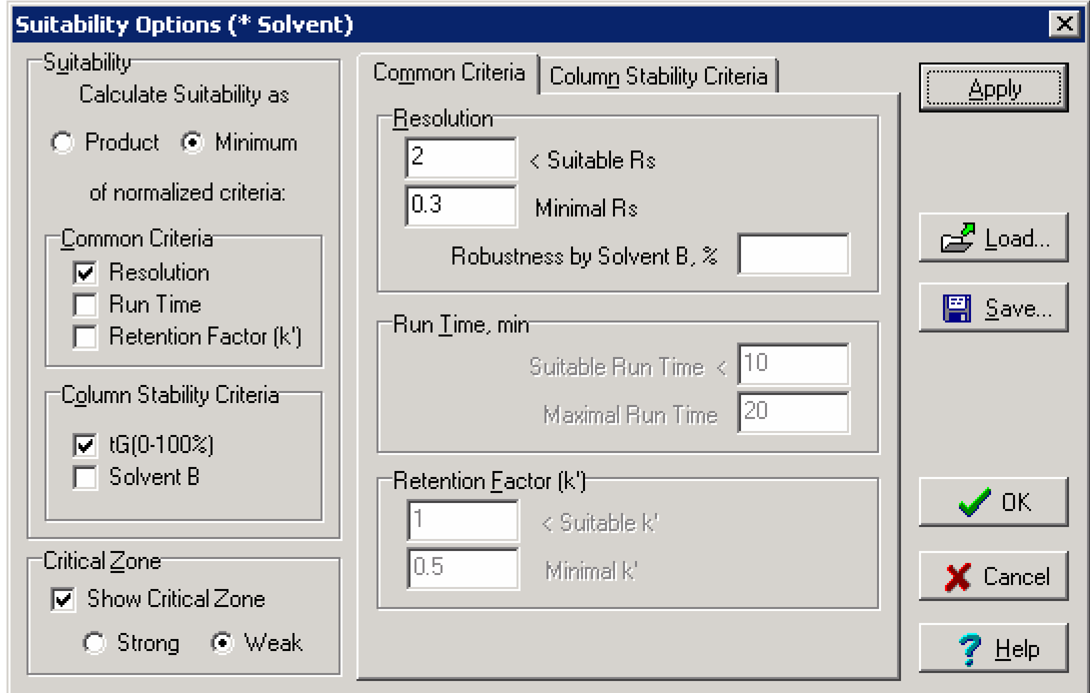
C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_9
4.54	4.15	4.73	5.51	1.13	4.41	4.14	5.07
4.53	4.15	4.73	5.51				
4.53	4.15	4.73	5.51				
4.53	4.15	4.73	5.51				
4.55			5.51	1.13	4.41	4.14	5.07
4.55			5.51	1.13	4.41	4.14	5.07
4.55			5.51	1.13	4.41	4.14	5.07
4.36	3.58	5.27	8.32		4.03	3.41	6.51
4.35	3.58	5.27	8.33				
4.35	3.58	5.27	8.33				
4.35	3.58	5.27	8.33				
4.37			8.3		4.03	3.41	6.51
4.37			8.3		4.03	3.41	6.51
4.37			8.3		4.03	3.41	6.51
5.99	5.29	6.25	7.62	1.13	5.76	4.92	7.03
5.99	5.29	6.25	7.63				
5.99	5.29	6.25	7.63				
5.99	5.29	6.25	7.63				
5.98			7.62	1.13	5.76	4.92	7.03
5.98			7.62	1.13	5.76	4.92	7.03
5.98			7.62	1.13	5.76	4.92	7.03

6. You are now ready to optimize conditions.

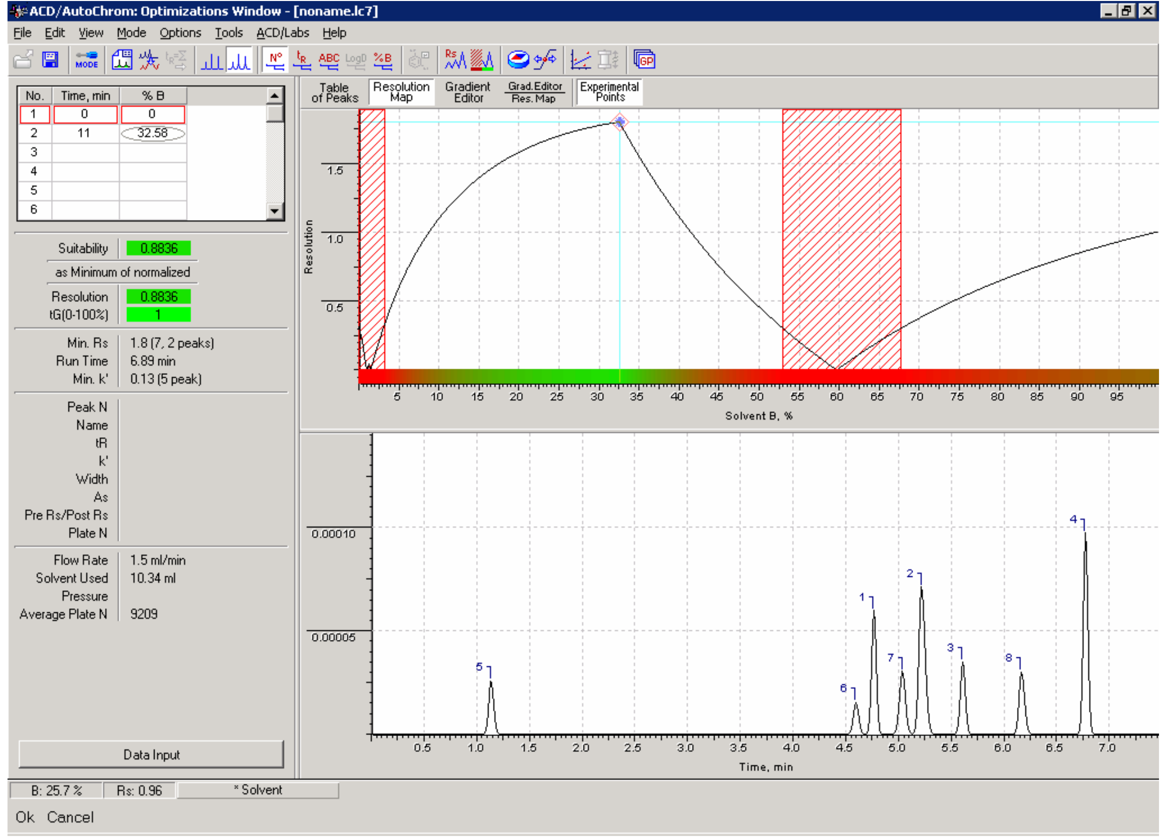
1.9 Searching for Optimum Conditions

1. On the **Assistant** tab, click accept these injections. You see more options presented.
2. Click optimize in ACD/LC Simulator. The program transfers all the processed files into LC Simulator, Optimization window.
3. Click **OK** to close the Modes Manager window.
4. You can see that all data are combined in the Solvent window.

5. Click **OK** to build a Resolution Map. Specify the Suitability options as shown below and you will see a green area on the Resolution Map.



6. Click **Search Optimal Conditions** to see the best separation.



7. Click **OK** on the bottom to accept these optimized conditions. You will be switched to the Task window and the new conditions will appear on the next Experiment line.

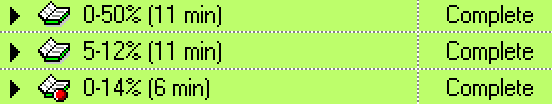
1.10 Completing the Project

1. Run a new experiment under the optimized conditions and bring data to the project by clicking start execution on the **Assistant** tab.
2. Take a look at the data file and, if you are satisfied, select this condition line on the **Assistant** tab and then click complete the current wave. If there were significant deviations between the predicted and experimental chromatograms, we could transfer the data to LC Simulator again, and rebuild the model. In this case, we will accept the results as-is.

How do we determine that the project is complete and see the best run in each wave?

In the Waves subwindow, navigate to the first wave (Column). In the Experiments subwindow, on the left toolbar, click **Show Experiments**. You will see only green lines and one line has a red dot which means this experiment is chosen as the best. In your case it is a run with the Luna C18 column.

In the Waves subwindow, navigate to the second wave (Optimization) and take a look at the experiment lines only. You will see that this wave is complete as well and a red dot present.



The project is complete, all data are very well represented, and you can now share this project with your colleagues or create a report.