

Gradient and Temperature Optimization

ACD/LC Simulator
Version 12.01




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Introduction

This technical note will describe the two dimensional optimization of gradient and temperature using ACD/LC Simulator¹. You will learn how to open data files, process the data (perform peak picking and peak matching), check and edit HPLC parameters, push data to LC Simulator, build a model, and choose the next run. We will also compare the predicted chromatogram with the experimental chromatogram.

This example data set consists of four LC/UV data files containing nine components of interest. The gradient is 5–95% B over 5 and 15 minutes and each gradient was acquired at 30°C and 60°C. The file names reflect the time and temperature of the experiment.


Opening the Data Files

1. On the far right of the **General Toolbar**, select **Full**  and **New Window Mode** .
2. From the **General Toolbar**, select **Open/Import** . In the **Import** dialog box, navigate to the location of the example data files listed below. Select them and click **Open**.

30deg_5min_DAD.esp
30deg_15min_DAD.esp
60deg_5min_DAD.esp
60deg_15min_DAD.esp

Processing the Data

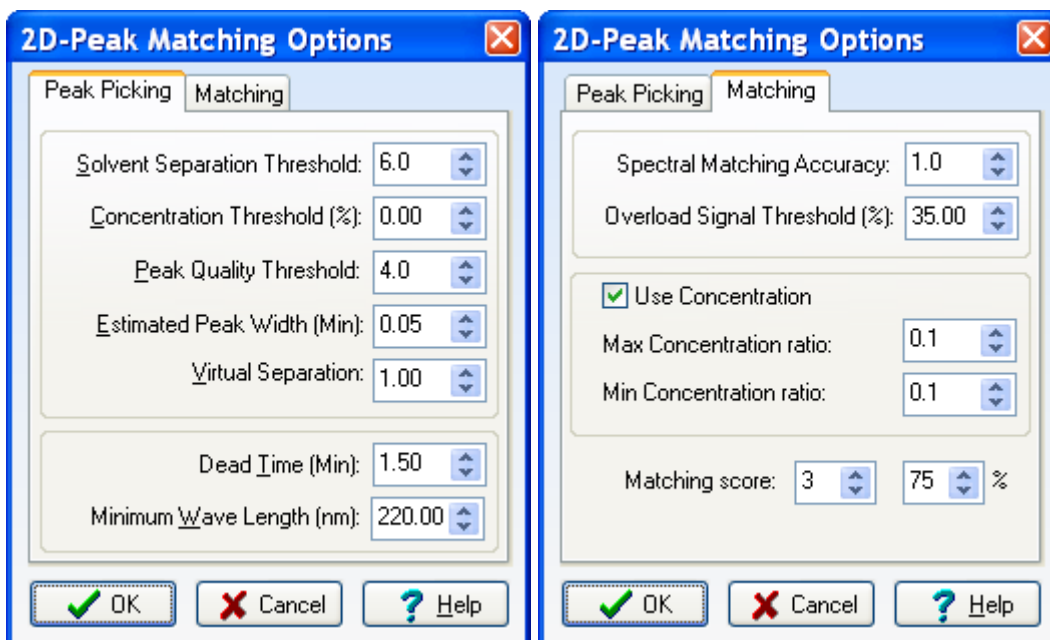
1. To process all of the data files together, from the **Windows** menu, choose **Select All**. Notice the data tabs are now yellow. The data file currently being viewed is orange.



Note The colors of the tabs may vary depending on your screen or settings applied.

2. From the **Operation toolbar**, select **Peak Matching**  to enter peak matching mode. Notice that the **Operation toolbar** changes depending on the mode you are in.

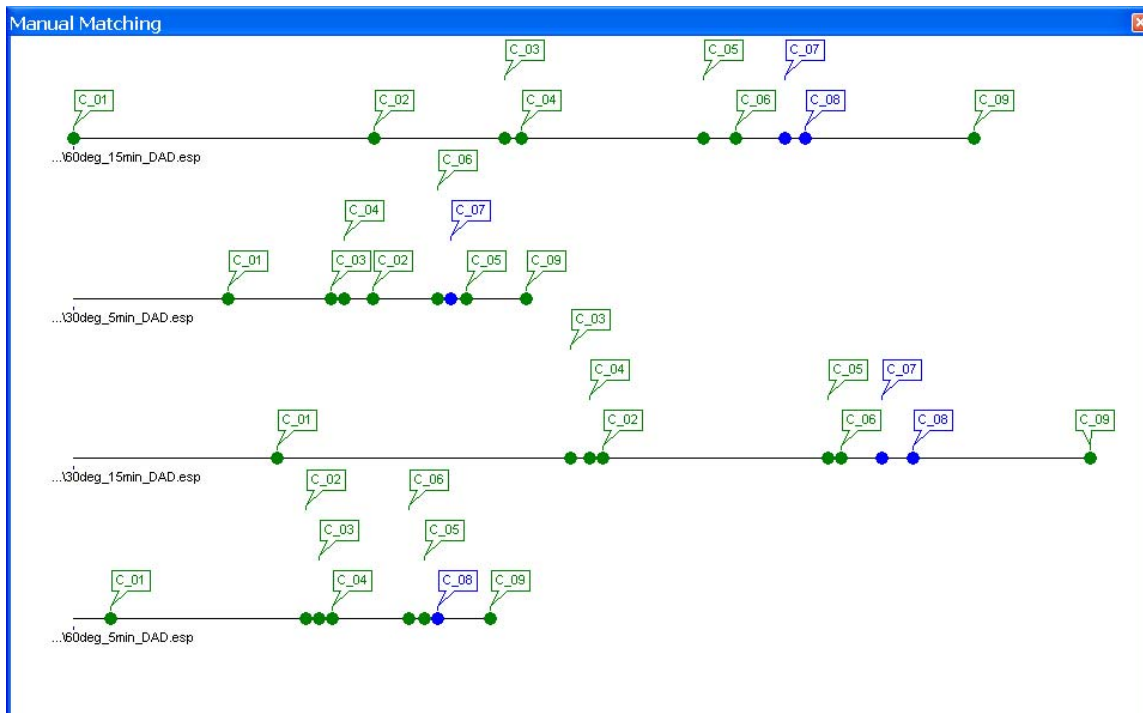
- From the **Operation toolbar**, select **Peak Matching Options** [Options...](#) to specify peak picking and peak matching options to use for this data set.
- Enter the following peak picking and peak matching parameters in the **2-D Peak Matching Options** dialog box and click **OK**.



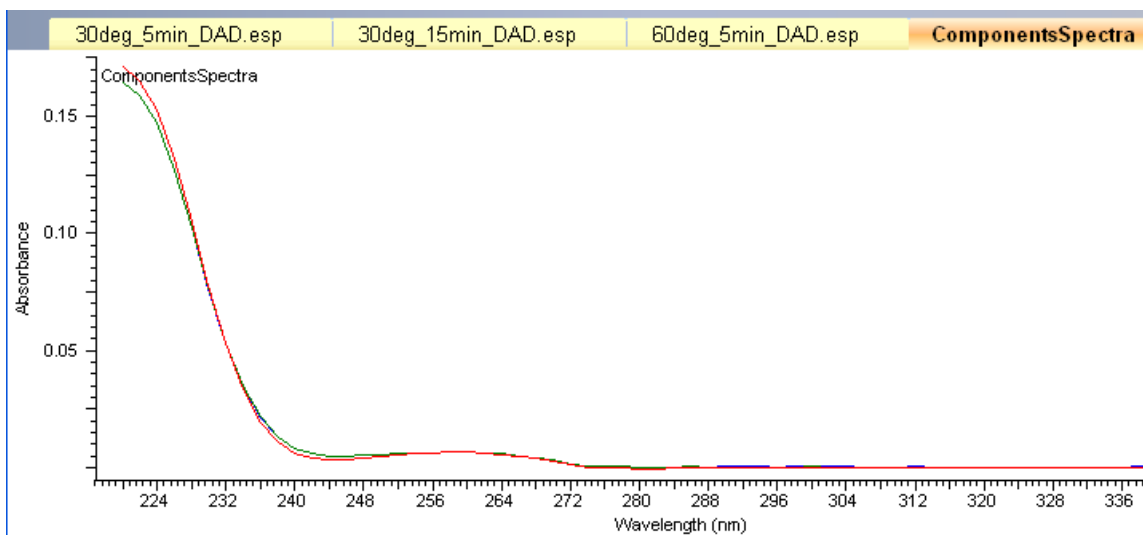
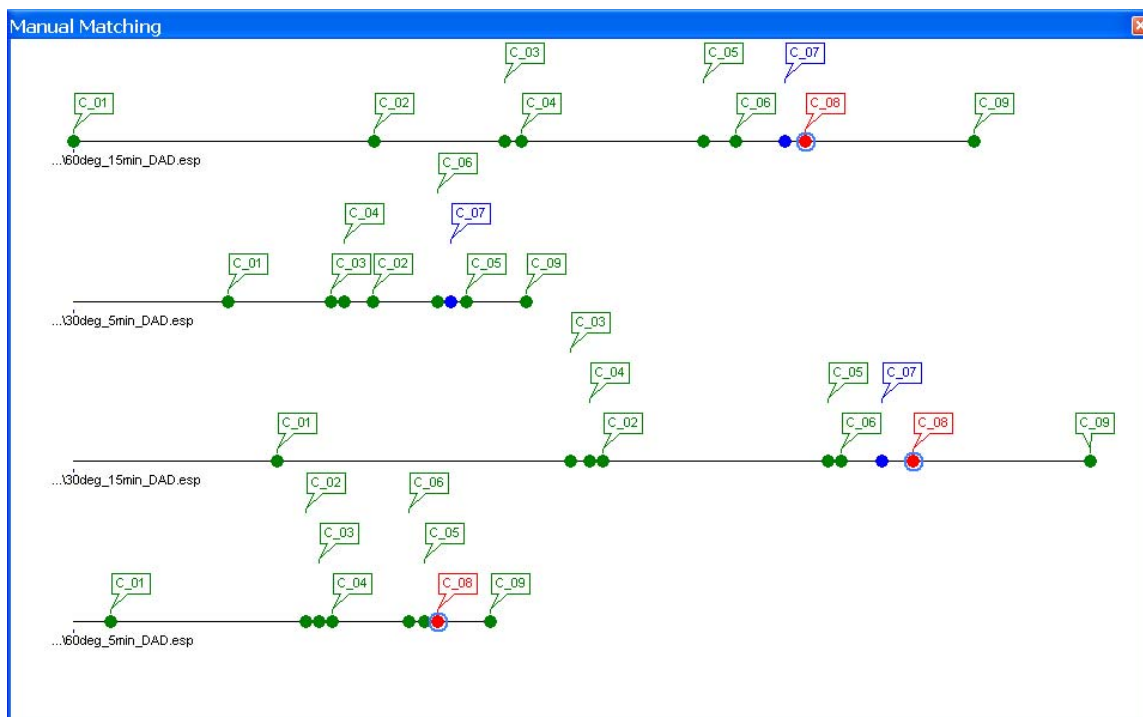
Note For detailed information about these parameters, refer to the *Introduction to UV-Mutual Automated Peak Matching (UV-MAP): How to Optimize the Input Parameters*² technical note found on our website at http://www.acdlabs.com/download/technotes/100/chrom/uv_peak_matching.pdf


- From the **Operation toolbar**, select **Match Selected Files** [Run](#) to apply the peak matching settings. Notice that the peaks are now labeled and matched.
- To take a closer look at the peak matching, select **Show Manual Matching Window** [Manual Matching](#). A new **Manual Matching** window and a **Components Spectra** tab appears.

7. In the **Manual Matching** window, each file (or chromatogram) is represented as a line and each component is represented as a dot on that line. A black dot represents a component found in only one file. A blue dot represents a component found in more than one file but, not in all of them. A green dot represents a component found in all files. In this example, C_07 and C_08 are blue dots meaning they are not found in all of the files. In the 30deg_5min experiment, C_08 is not found and appears to co-elute with C_05. In the 60deg_5min experiment, C_07 is not found and appears to co-elute with C_05 also.



- In the **Components Spectra** tab, you can view the spectra of the components. To do this, click on a dot, the component is highlighted in red and the spectrum for that component is displayed. To overlay several components spectra at once, hold down CTRL and click other dots. To overlay all spectra at once for a given component across all files, hold down SHIFT and click the dot.



- When you are finished comparing the data files, close the **Manual Matching** window.
- Click **Accept Changes**  to accept the changes and exit peak matching mode.

Export TIC to ChromManager

So far, we have been working with LC/UV data files for processing. In order to transfer the data to LC Simulator for optimization, we need to work with non-hyphenated data files (also referred to as flat chromatograms).

1. From the **Chromatogram** menu, choose **Export TIC to ChromManager**. This will create a new data file tab next to the selected data file with _TIC added to the file name.

30deg_5min_DAD.esp 30deg_5min_DAD_TIC

2. Repeat this step for each data file.
3. Now, we no longer need the LC/UV data files and can close them. With one of the LC/UV data files selected, from the **File** menu, choose **Close**.
4. Close all of the hyphenated data files leaving only the data files with _TIC in the file name open.

30deg_5min_DAD_TIC 30deg_15min_DAD_TIC 60deg_5min_DAD_TIC 60deg_15min_DAD_TIC

Editing HPLC Parameters

Before transferring this data to LC Simulator for optimization, we need to input the HPLC parameters. Some of the parameters are pre-populated depending on the type of data and how the data was imported. Certain parameters such as dwell volume, dead time, and flow rate are required parameters for optimization.

1. From the **General Toolbar**, select **Edit/Show Chromatogram Parameters** .

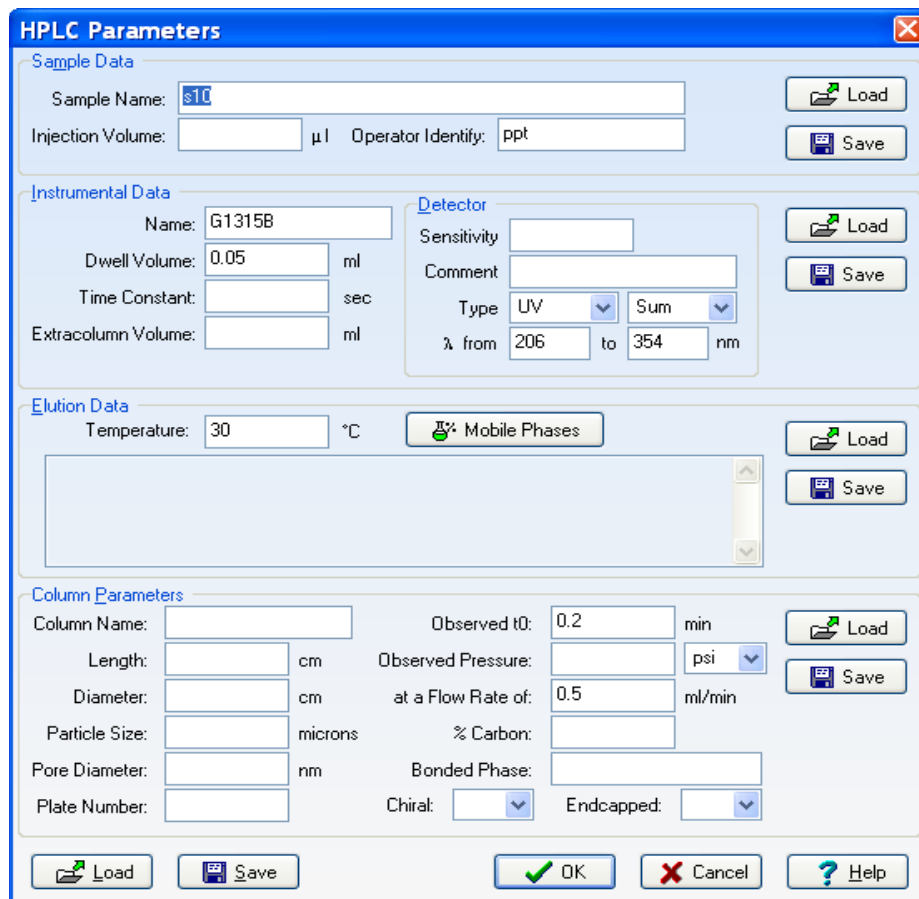
2. Input the following parameters in the **HPLC Parameters** dialog box:

Dwell Volume = 0.05 ml

Observed t₀ = 0.2 min

Flow Rate = 0.5 ml/min

Temperature = 30°C or 60°C depending on which data file is selected



HPLC Parameters

Sample Data

Sample Name:

Injection Volume: μl Operator Identify:

Instrumental Data

Name:

Dwell Volume: ml

Time Constant: sec

Extracolumn Volume: ml

Detector

Sensitivity:

Comment:

Type: UV

λ from: to: nm

Elution Data

Temperature: °C

Column Parameters

Column Name:

Length: cm

Diameter: cm

Particle Size: microns

Pore Diameter: nm

Plate Number:

Observed t₀: min

Observed Pressure: psi

at a Flow Rate of: ml/min

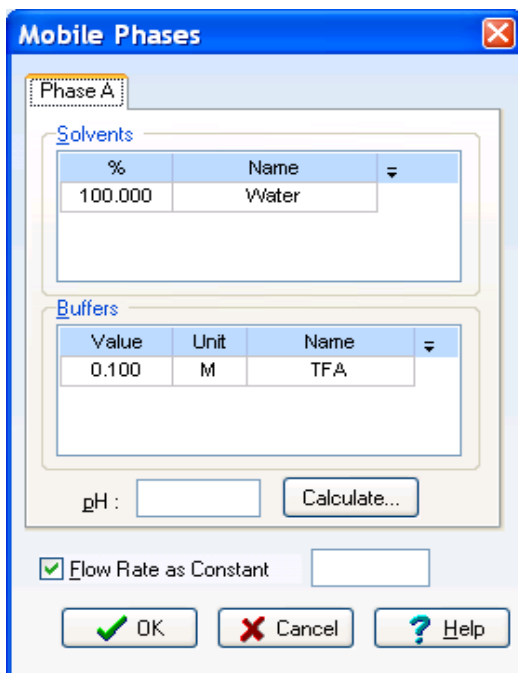
% Carbon:

Bonded Phase:

Chiral: Endcapped:

3. In the **HPLC Parameters** dialog box, click **Mobile Phases** to input the gradient program.
4. In the **Mobile Phases** dialog box, right-click in the **Solvents** box, and choose **Add**. Input 100 in the % column and **Water** in the **Name** column for **Solvent A**.

- Right click in the **Buffers** box, and choose **Add**. Input 0.1 M TFA as the **Buffer** for **Solvent A**.



Mobile Phases

Phase A

Solvents

%	Name
100.000	Water

Buffers

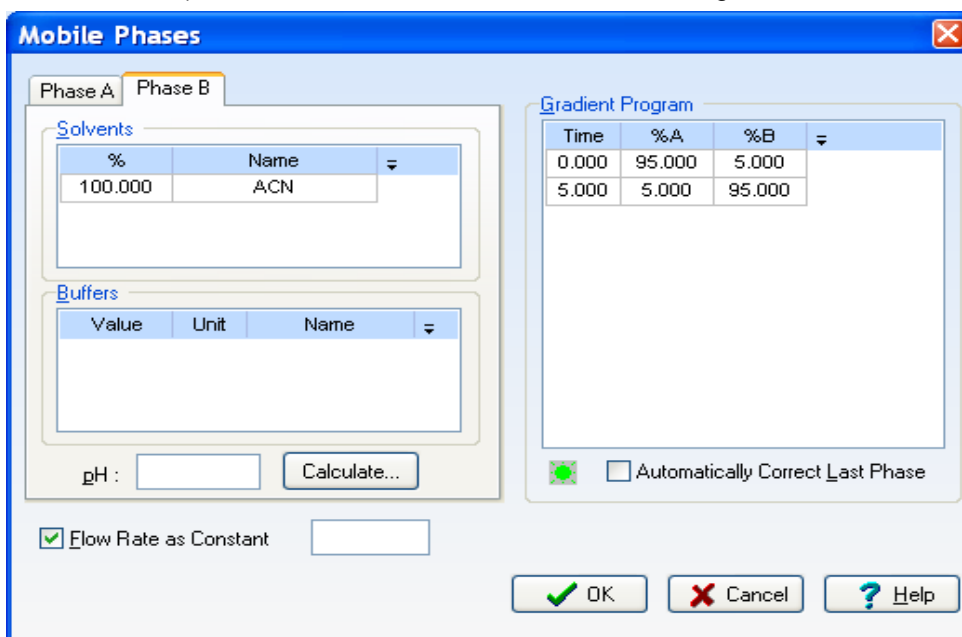
Value	Unit	Name
0.100	M	TFA

pH : Calculate...

Flow Rate as Constant

OK Cancel Help

- Right-click on the **Phase A** tab and choose **Add**. The **Phase B** tab is added and the **Gradient Program** box is displayed.
- On the **Phase B** tab, in the **Solvents** box, input 100% ACN for **Solvent B**.
- Right-click in the **Gradient Program** box and choose **Add**. Add two lines to the gradient program box and input the gradient program. (5 or 15 minute gradient depending on the data file that is selected.) Click **OK** to close the **Mobile Phases** dialog box.



Mobile Phases

Phase A Phase B

Phase A Solvents

%	Name
100.000	ACN

Phase B Solvents

%	Name
---	------

Gradient Program

Time	%A	%B
0.000	95.000	5.000
5.000	5.000	95.000

Automatically Correct Last Phase

pH : Calculate...


Flow Rate as Constant

OK Cancel Help

- Click **OK** to close the **HPLC Parameters** dialog box.
- Highlight the next data file and repeat these steps to enter the **HPLC Parameters** for the other data files.

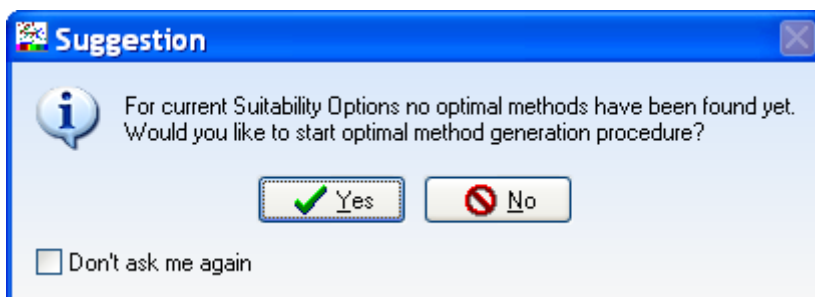
Transferring to LC Simulator/Building the Model


We are now ready to transfer this data to LC Simulator for optimization.

- With all of the data files still selected, click on the first file.
- From the **General Toolbar**, select **Transfer to LC Simulator** .
- In the **Select Mode** dialog box, select **RP Gradient/Temperature**.
- All of the experiments are summarized in the **RP Gradient/Temperature** dialog box in the **Combined** tab. Notice the holes in the table where C_07 and C_08 are not found. LC Simulator excludes components from the model that are not found in all of the experiments.
- In order to include C_07 and C_08 in the model, we have to manually input the retention time in the table. As mentioned previously, (in number 8 of the Processing the Data section) C_07 and C_08 co-elute with C_05. Double-click in the empty cells and input the following retention times:

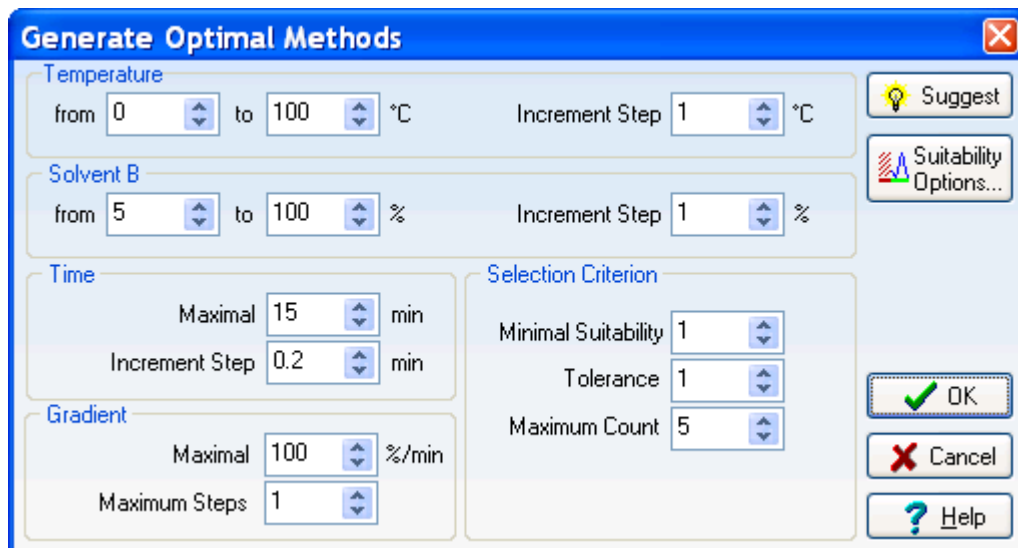
7	NO	✓	C_07	5.94	4.07	6.49	3.94
8	NO	✓	C_08	6.06	4.16	6.66	3.99

- Click **OK**.
- The following **Suggestion** dialog box appears stating that we do not have an optimal method with the current **Suitability Options**. Click **Yes** to start the optimal method generation procedure and to change the **Suitability Options**.

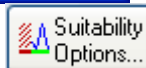


Note If you do not see this message, it may be because your suitability options are different. If the **Suggestion** box does not pop up, from the **General Toolbar**, select **Generate Optimal Methods** .

8. In the **Generate Optimal Methods** dialog box, you can specify certain limitations (such as Temperature, % B, and Time) or have the program suggest them for you based on the **Suitability Options**. We are going to specify the **Suitability Options** first and have the software suggest these settings.

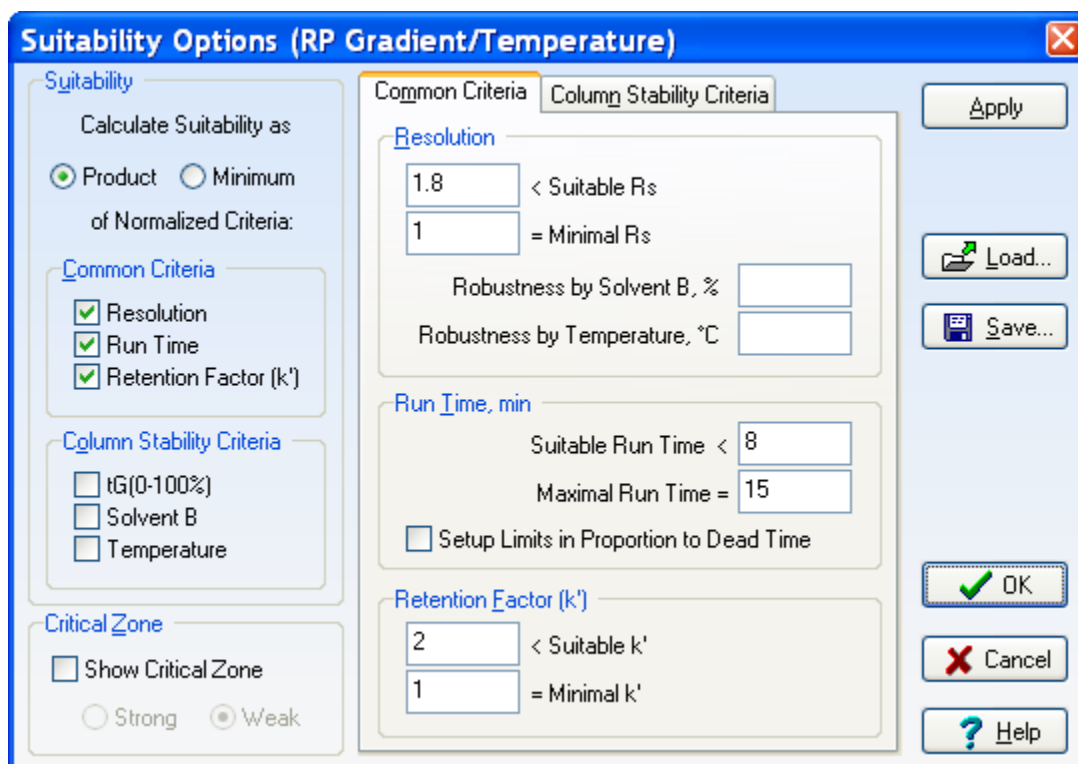



9. In the **Generate Optimal Methods** dialog box, click on **Suitability Options**

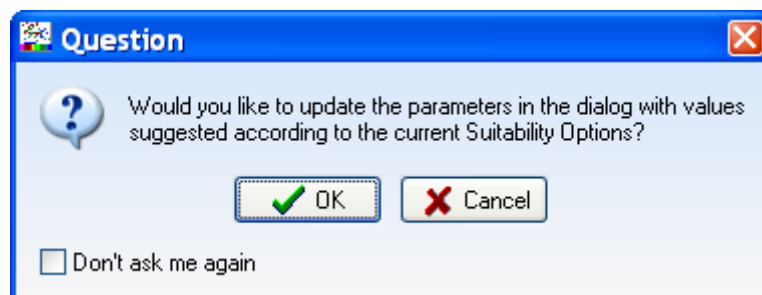


Note The **Suitability Coefficient** is a numerical value from 0 to 1. Suitability is calculated as the minimum or product of separate normalized criteria: resolution (with robustness), run time, retention factor, and column stability parameters. For each criterion, you can specify the desired and permissible interval of values. Normalized criterion is equal to: 1 inside desired interval, 0 outside the permissible interval, and is calculated linearly from 0 to 1 in other cases. On the resolution map, the suitability is expressed through colors from red (0) to green (1).

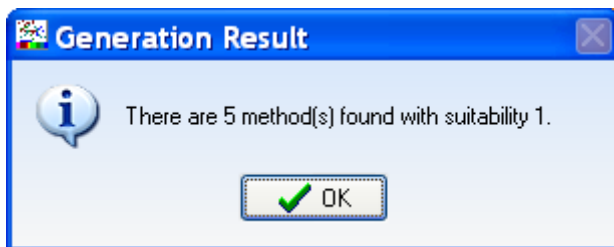
10. In the **Suitability Options** dialog box, specify the following options. In this example, we are calculating suitability as a product of resolution (minimal resolution is 1, suitable resolution is greater than 1.8), run time (maximal run time is 15 minutes, suitable run time is less than 8 minutes), and retention factor (minimal k' is 1, suitable k' is greater than 2) and have specified acceptable intervals for each. Resolution and run time are the most important criteria for suitability. If you specify the suitability options and optimal conditions cannot be found, you probably need to change the criteria values for resolution or run time or both.



11. Click **OK** in the **Suitability Options** dialog box.
12. The following **Question** box appears, asking if we want to update the parameters in the **Generate Optimal Methods** dialog box based on the **Suitability Options** we just specified, click **OK**. (Clicking OK here is the same as choosing **Suggest**  **Suggest** in the **Generate Optimal Methods** dialog box.)

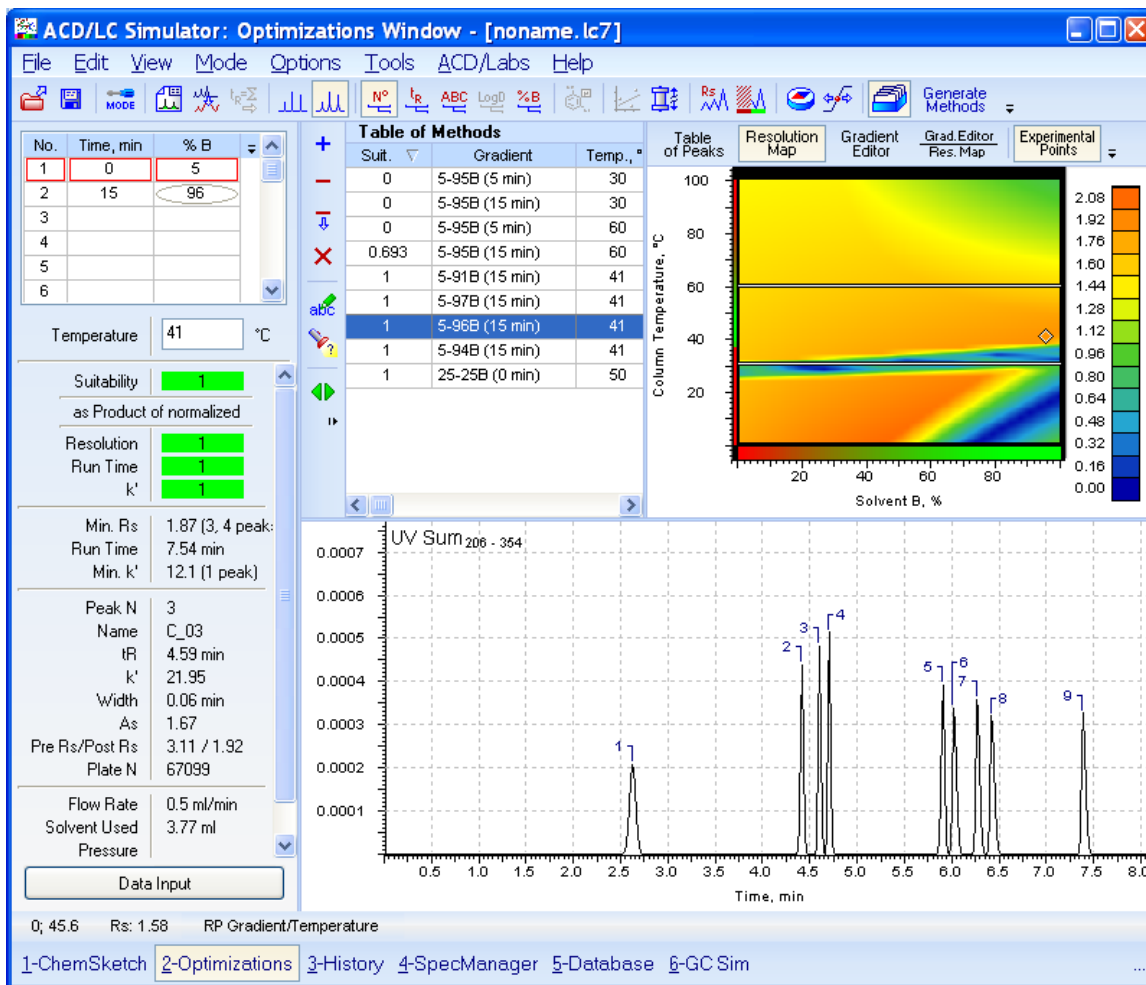


- Click **OK** in the **Generate Optimal Methods** dialog box.
- The **Generation Result** dialog box appears and states that we have five methods found with a suitability value of 1. Click **OK** and the resolution map is calculated.



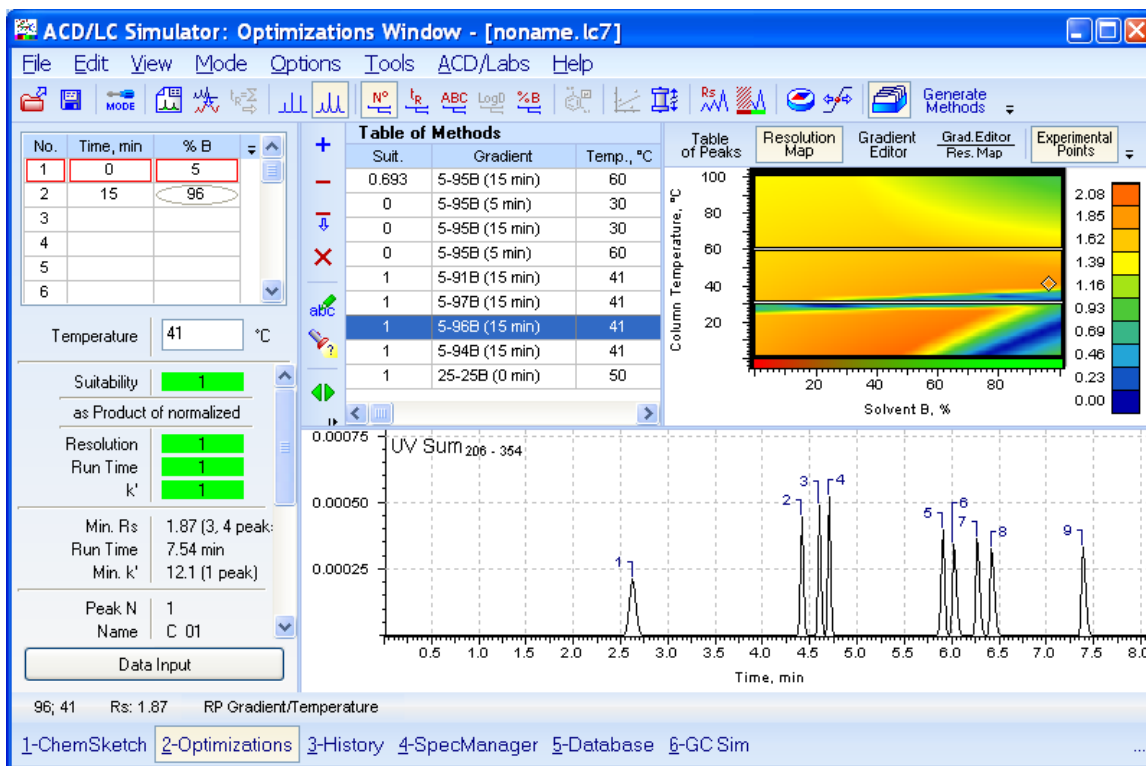
Note If you want the program to find more than five methods, in the **Generate Optimal Methods** dialog box, in **Selection Criteria**, increase the **Maximum Count**.

- Those five methods and the original methods are displayed in the **Table of Methods**.




Choosing the Next Run

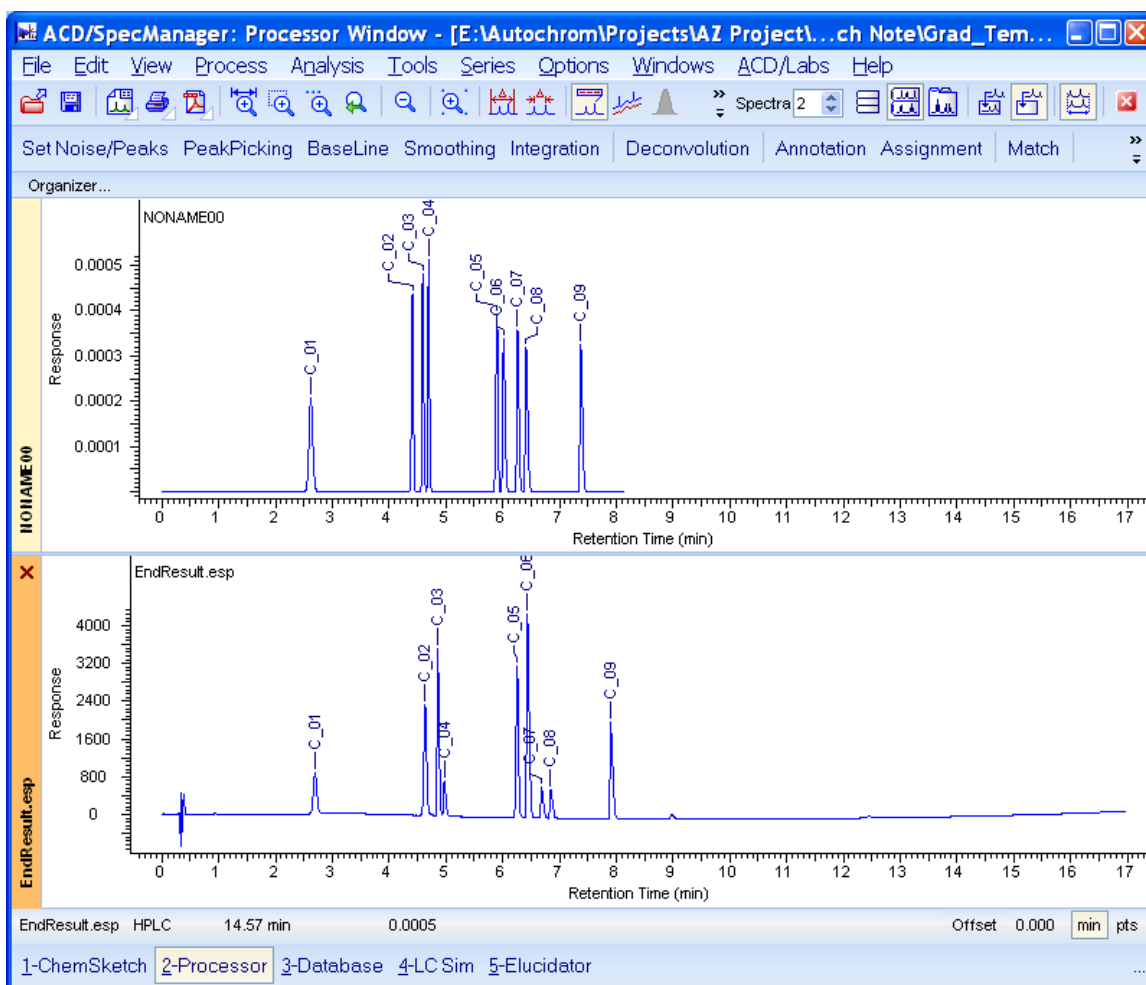
The model has been created and now we need to determine which method to choose for our next run. If you click on each new method in the **Table of Methods**, the resolution map, chromatogram and other parameters (min Rs, run time, and min k', etc.) are recalculated for each method. We can choose any of the five methods since they all have a suitability value of 1. For this example, choose the following method: 5–96 %B in 15 minutes at 41°C.



Predicted vs. Experimental Chromatograms

Now, let's compare the predicted chromatogram with the experimental chromatogram.

1. From the **Edit** menu, choose **Copy to SpecManager**. This will transfer the predicted chromatogram that we have selected in ACD/LC Simulator to ACD/SpecManager³.
2. In **SpecManager**, open the EndResult.esp data file also provided with this technical note.
3. On the far right of the **General Toolbar**, select **Tile view** .
4. From the **Windows** menu, choose **Select All**.
5. From the **Windows** menu, choose **Synchronize**.



Conclusion

This technical note describes the workflow for gradient and temperature optimization using ACD/LC Simulator. We started with four raw LC/UV data files and peak picked and matched the data then transferred it to LC Simulator. Then, we built a model based on suitability criteria that we specified and determined our next experiment. From the comparison of predicted vs. experimental chromatograms, we can see that the end results from the gradient and temperature optimization are accurate.

References

1. ACD/LC Simulator. www.acdlabs.com/lcsim/ Advanced Chemistry Development, Inc., Toronto, ON, Canada. July 20, 2009.
2. Technical Note: *Introduction to UV-Mutual Automated Peak Matching (UV-MAP): How to Optimize the Input Parameters*, July 20, 2009. http://www.acdlabs.com/download/technotes/100/chrom/uv_peak_matching.pdf
3. ACD/SpecManager. www.acdlabs.com/specmanager/ Advanced Chemistry Development, Inc., Toronto, ON, Canada. July 20, 2009.