

CHM 416/1104 Virtual Chromatography Laboratory Instructions

Part 2—The Effect of Solvent Strength on Separations

In this exercise, you will be using ACD/Labs 7.0 software to examine the effects of solvent gradient on chromatographic behavior. In this particular case, you will make use of chromatograms that have already been combined and prepared for use in the LC Simulator module. ACD/LC Simulator will use the results from two different gradient experiments to predict the results for any gradient that you design, including an isocratic separation where no solvent gradient is used.

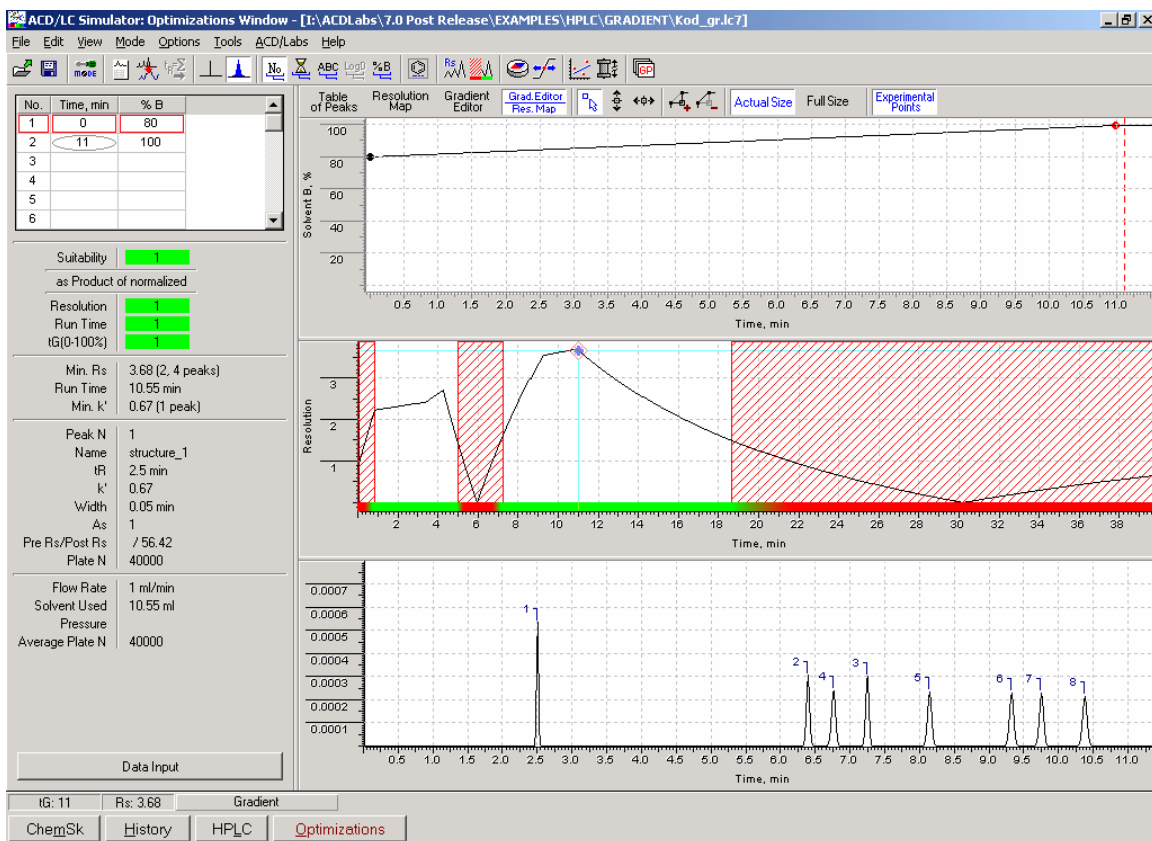
Introduction

The optimization algorithms in LC Simulator take advantage of the empirical relationship between solvent strength and k' . In general, $\log k'$ can be expected to drop in proportion to the solvent strength (%B). The exact dependence in a given system is a function of each individual compound, and this variability is the main reason that changes in solvent strength can be used to get changes in resolution.

A resolution map is a useful way to visualize the effect of a given variable on the separation of a set of compounds. Resolution maps describe the resolution of the critical pair (least-resolved components) as a function of a given variable.

Exercise 2: Exploring Gradient Conditions

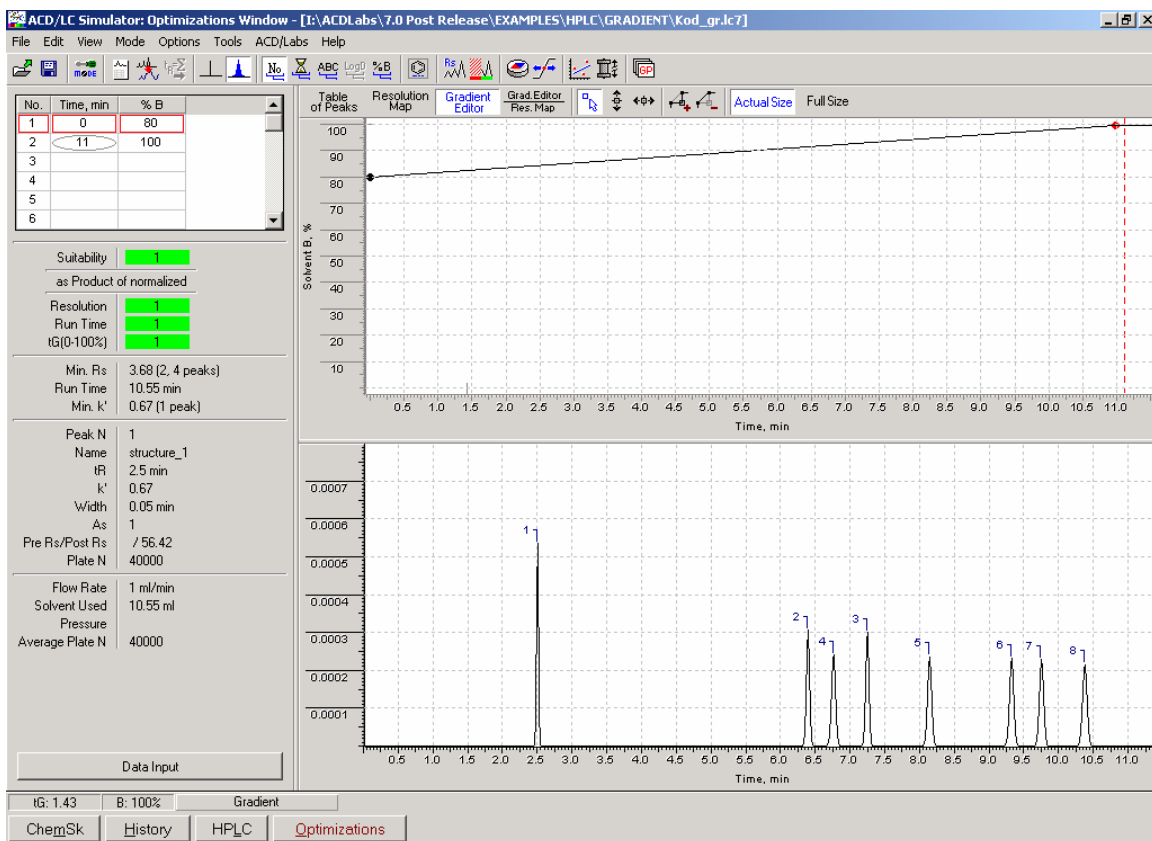
- 2.1 From the **Start** menu, point to **Programs**, then point to **ACDLabs7.0**, and then choose **ACD LC Simulator**.
- 2.2 Open the file "C:\ACD70TL\Examples\HPLC\Gradient\Kod_gr.lc7".



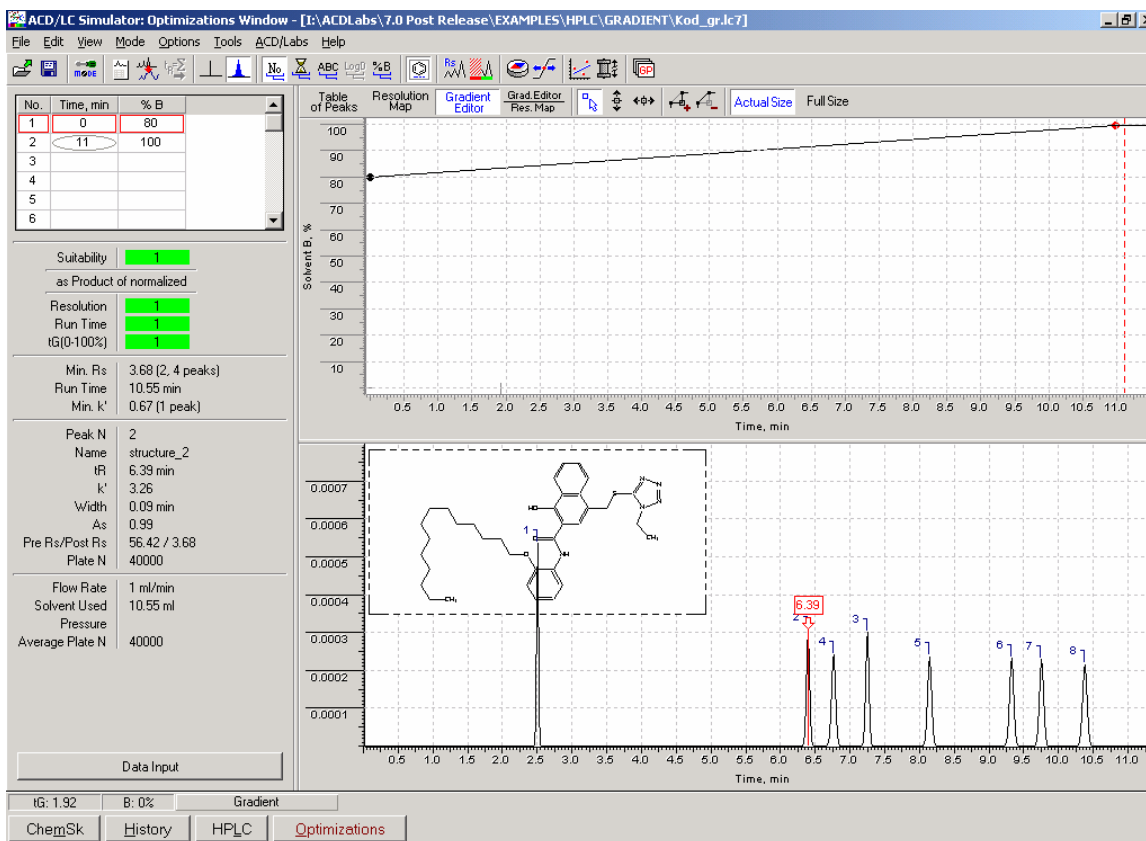
Important: Please do **not** save any changes you make back to the original file. If you need to interrupt your work, save the modified file with a unique file name.

2.3 Navigating through the LC Simulator Module:

You will notice that the file opens with various graphical panels on the right-hand side of the screen. You can vary which panels are visible through the **View** menu items or the corresponding buttons on the second toolbar (located directly above the plots). In particular, you can use the **Gradient Editor** button/item to see the graph of the solvent gradient in more detail.



You can also turn the display of the peak structures on and off; from the **View** menu, point to **Structure Window**, and then choose **Show** or **Floating Window**.



Finally, you can vary the way in which the peaks are labeled between peak numbers, retention times, and $\log D$ values; from the **View** menu, point to **Labels**, and then choose the desired option or click the corresponding buttons in the main tabular display of the current gradient conditions in the top-left of the main window, and a summary of various related parameters below this.

- 2.4 Click **Data Input** (lower left-side of the main window). You will notice that the resulting **Gradient** dialog box has four tabbed panes, consisting of the HPLC parameters, the two experimental chromatograms on which all predictive calculations are based, and a “combined” chromatogram. In the **HPLC Parameters** pane, click **Mobile Phases** and notice the identity of the solvents used for this gradient.

Gradient 7.0

HPLC Parameters | Combined | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th

Instrumental Data

Name:

Dwell Volume: ml

Time Constant: sec

Extracolumn Volume: ml

Detector

Sensitivity:

UV RI Other

$\lambda =$ nm

Elution Data

Temperature: °C

Flow Rate : 1 ml/min
Phase A : Water
Phase B : MeCN

Column Parameters

Column Name:

Length: cm

Diameter: cm

Particle Size: microns

Pore Diameter: nm

Plate Number:

Observed t₀: min

Observed Pressure: bar

at a Flow Rate of: ml/min

Endcapped:

% Carbon:

Bonded Phase:

Also, look in the panes for the two experimental chromatograms.

Gradient 7.0

HPLC Parameters Combined 1st 2nd 3rd 4th 5th 6th 7th 8th 9th

structure_1 Width As Area %Area

No	Struc	Name	tR	Width	As	Area	% Area
1	YES	structure_1	2.50				
2	YES	structure_2	6.30				
3	YES	structure_3	7.10				
4	YES	structure_4	6.70				
5	YES	structure_5	8.00				
6	YES	structure_6	9.10				
7	YES	structure_7	9.50				
8	YES	structure_8	10.10				

Isocratic Gradient

Experimental Parameters

Time: min

No	Time	% B
1	0.000	80.000
2	10.000	100.000
3		
4		
5		

Gradient 7.0

HPLC Parameters Combined 1st 2nd 3rd 4th 5th 6th 7th 8th 9th

structure_1

Width As Area %Area

No	Struc	Name	tR	Width	As	Area	% Area
1	YES	structure_1	2.00				
2	YES	structure_2	4.40				
3	YES	structure_3	5.10				
4	YES	structure_4	5.60				
5	YES	structure_5	6.50				
6	YES	structure_6	7.50				
7	YES	structure_7	7.90				
8	YES	structure_8	8.00				

Isocratic Gradient

Experimental Parameters

Time: min

No	Time	% B
1	0.000	85.000
2	10.000	100.000
3		
4		
5		

Load Save OK Cancel Help

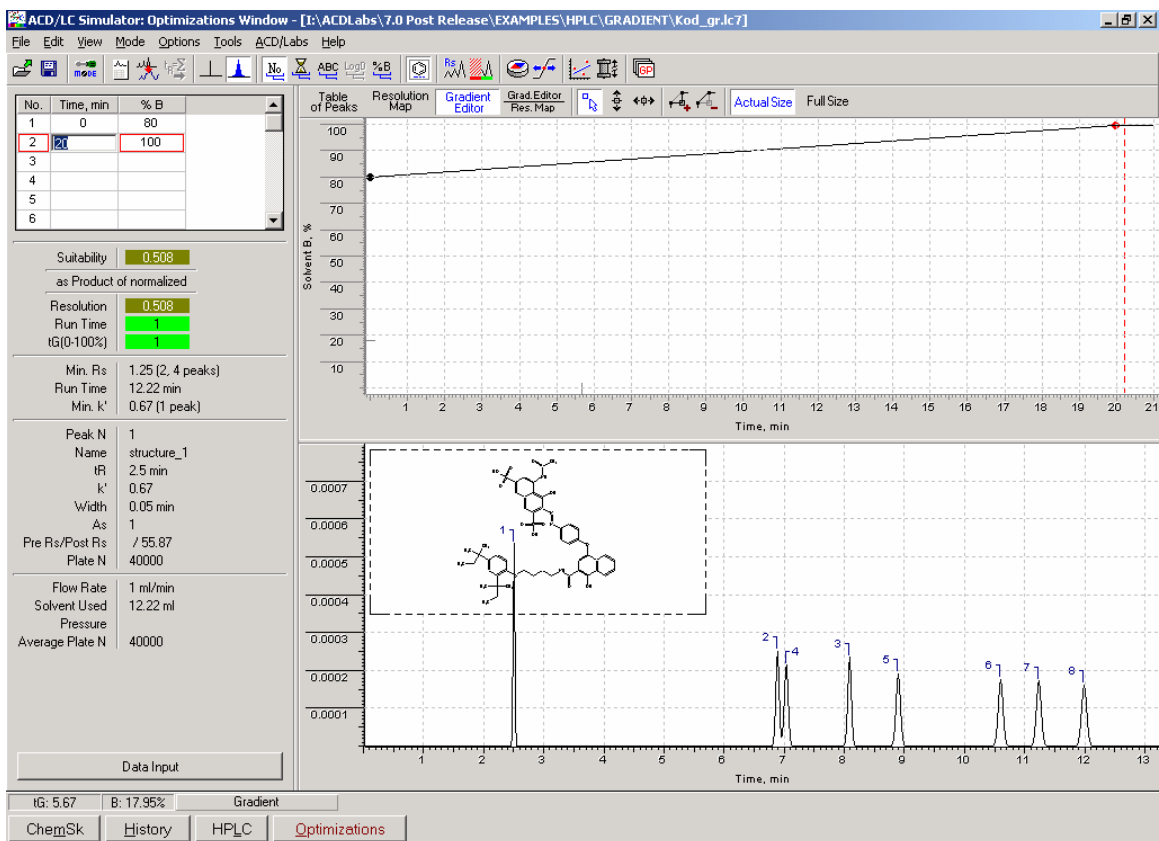
Q1. What were the two solvent gradients used in the two experimental chromatograms?

Q2. What type of HPLC is being performed—normal or reversed phase?

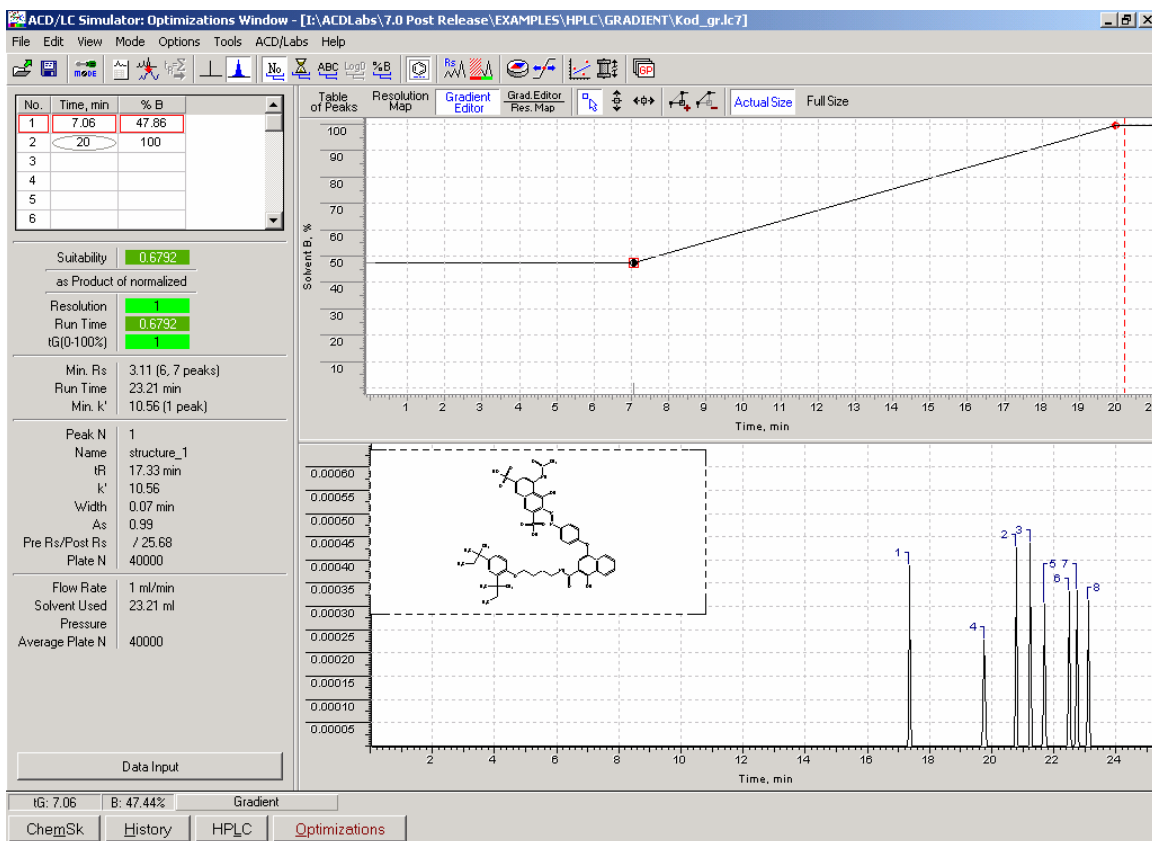
Close the **Gradient** dialog box.

2.5 You can vary the gradient conditions in the main LC Simulator window in two different ways:

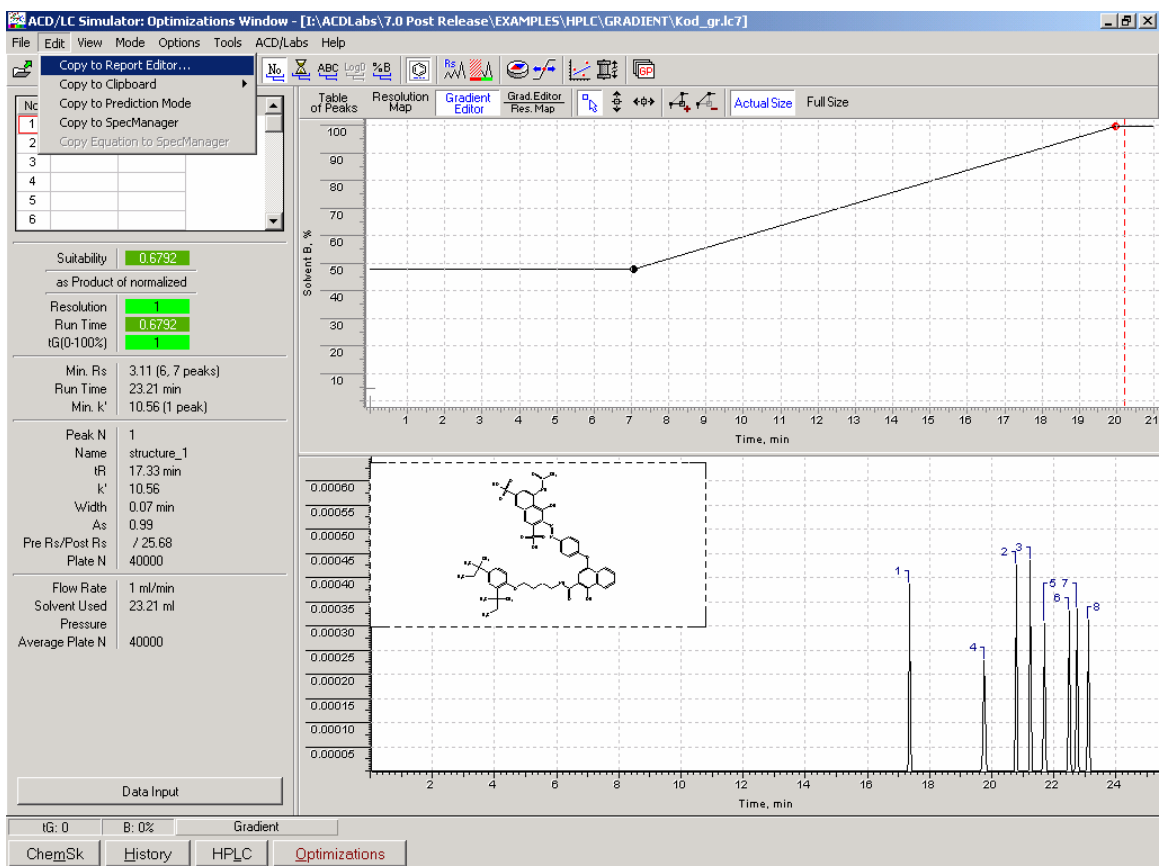
- (a) Manually, by entering values into the “spreadsheet” in the upper-left corner of the window and pressing ENTER.



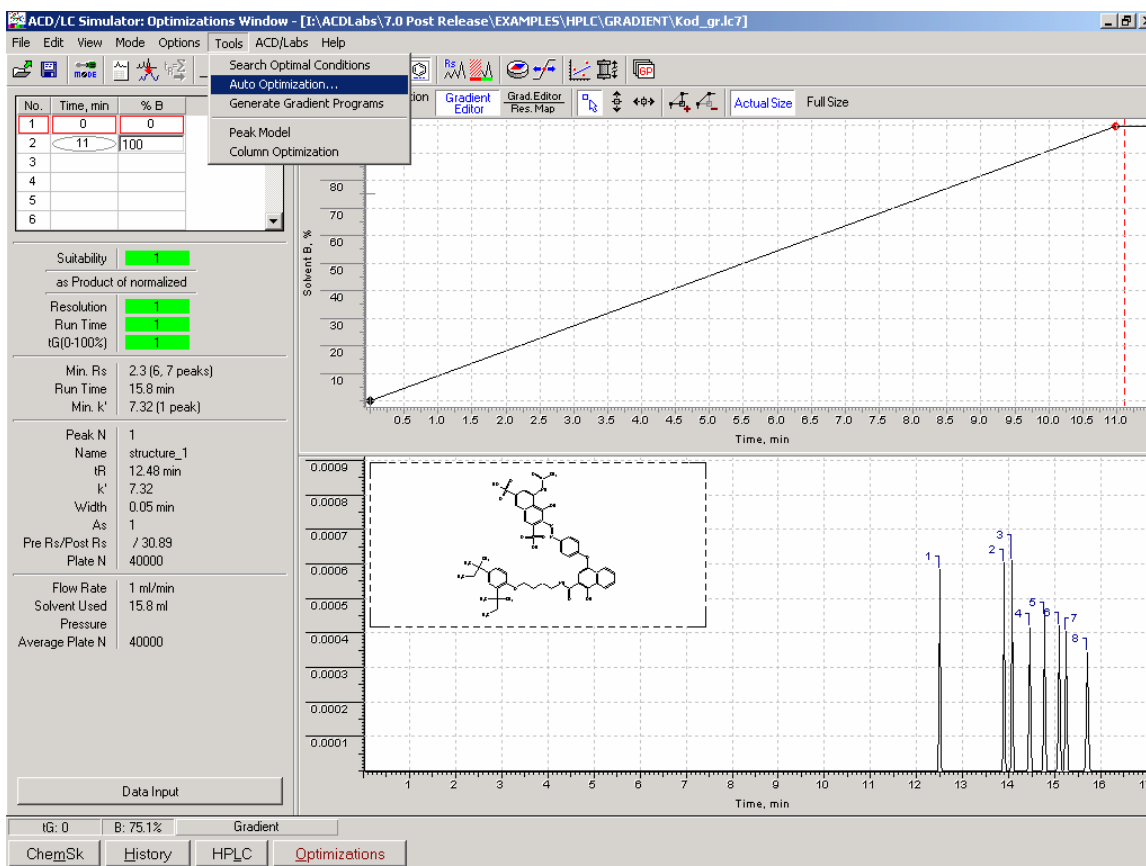
- (b) By clicking and dragging the gradient points (filled circles) within the gradient map display. Note that you can restrict movement to either horizontal or vertical, or revert motion in both directions, using the three buttons grouped together in the toolbar directly above the gradient map.



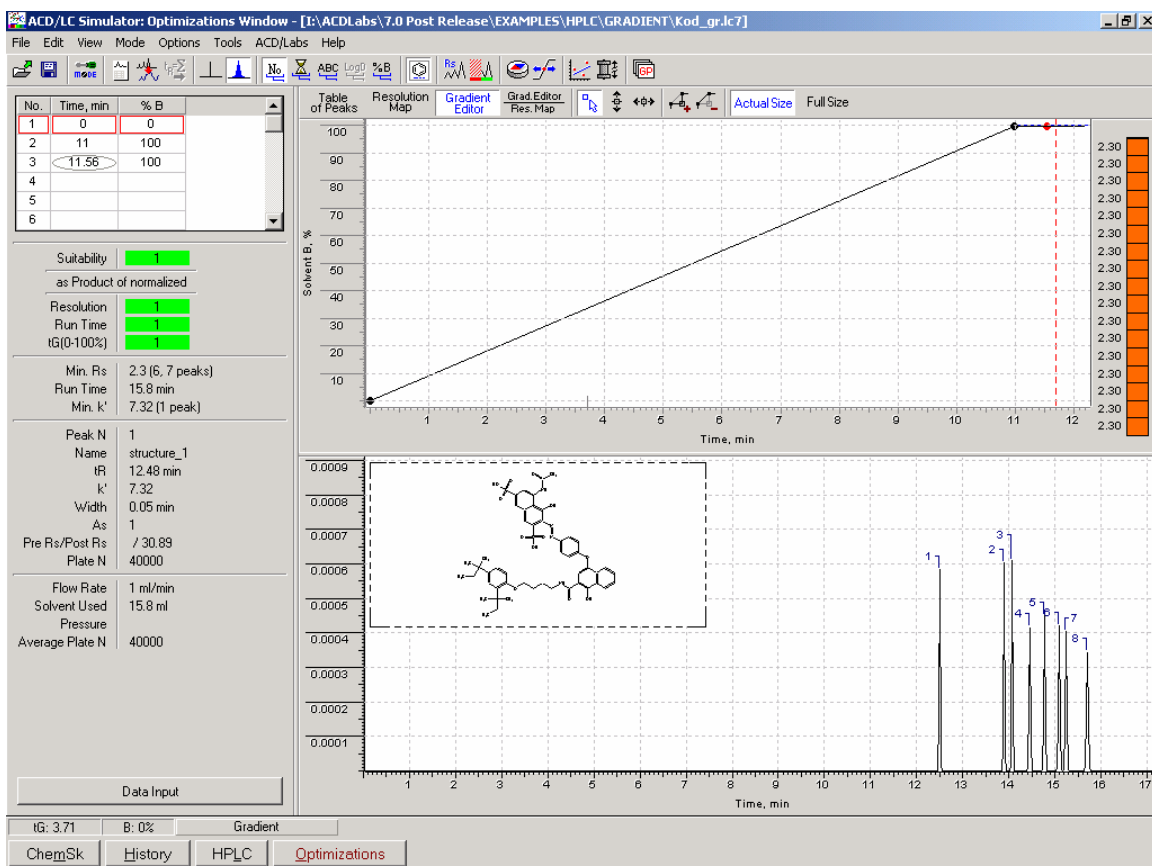
- Q3. Compare the effect of using a solvent gradient of 0-100% B over the time range 0-11 minutes). What do you notice about: the analysis time? The elution order? Resolution within the chromatogram?
- Q4. Given your answers to Q3, do you think a better compromise between resolution and analysis time can be obtained by having a lower or higher initial %B?
- 2.6 Manually vary the initial %B until you obtain a chromatogram you feel provides a good compromise between resolution and analysis time. To print a report, from the **Edit** menu, choose **Copy to Report Editor** and then print from ChemSketch. Include this report with your answers for this exercise.



- Q5. Summarize any changes you observed as you varied the gradient. Did your results confirm or contradict your answer to Q4?
- Q6. Using the data from the report you generated in this step, calculate the resolution, capacity factors (k') and selectivity ratio (α) for the two closest peaks.
- 2.7 The simulation module provides some tools to automate optimization of the gradient conditions, primarily through changing the number and position of gradient points. First, reset the solvent gradient to be 0% B at 0 minutes and 100% B at 11 minutes. Then from the **Tools** menu, choose **Auto Optimization** (or the corresponding toolbar button).



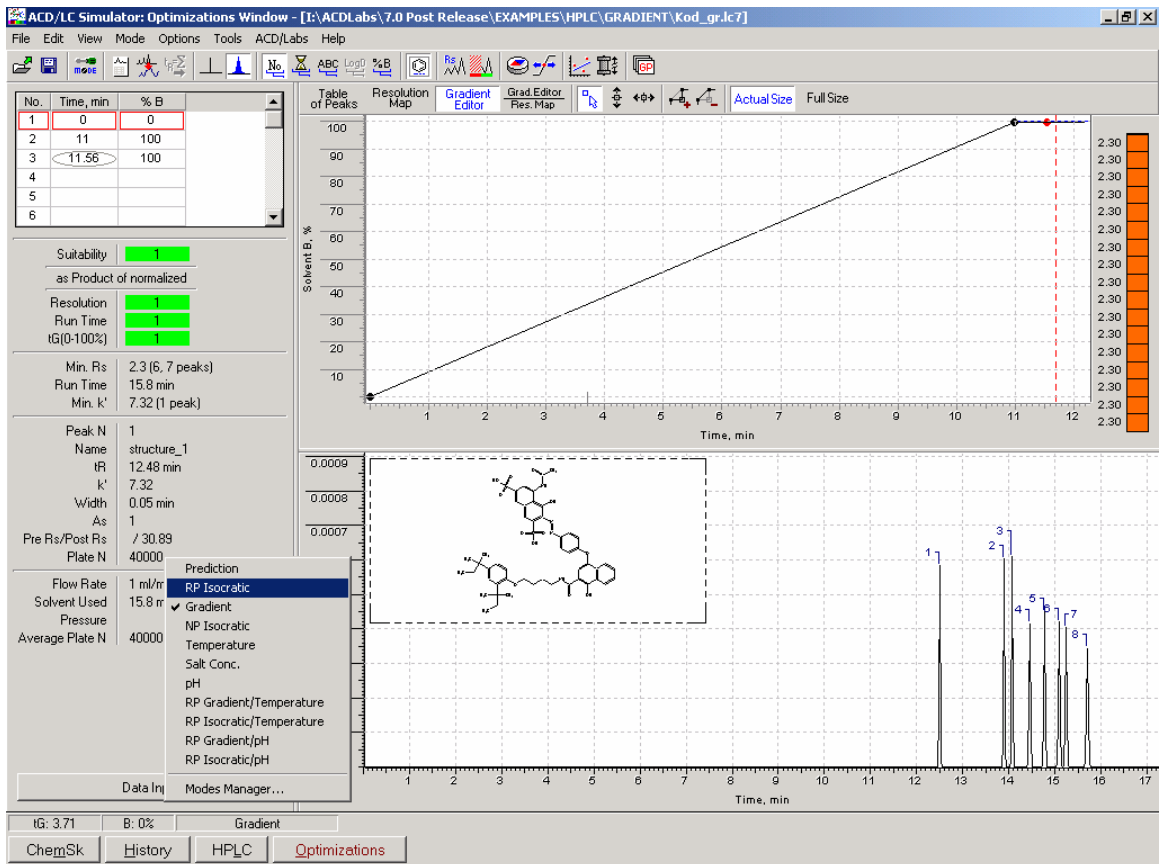
- 2.8 In the dialog box that appears, under **Optimization**, select **Point Position** and click **OK**. Generate and print a copy of the report as previously described and include this with your answers.
- Q7. What happens to the gradient and the overall chromatogram? How does this compare to the result you obtained in step 2.7?
- 2.9 From the **Tools** menu, choose **Auto Optimization** (or the corresponding toolbar button). In the dialog box that appears, under **Optimization**, select **Point Addition**, check that the **Last Segment** option is selected, and then click **OK** in both dialog boxes. This adds an additional point to the solvent gradient.

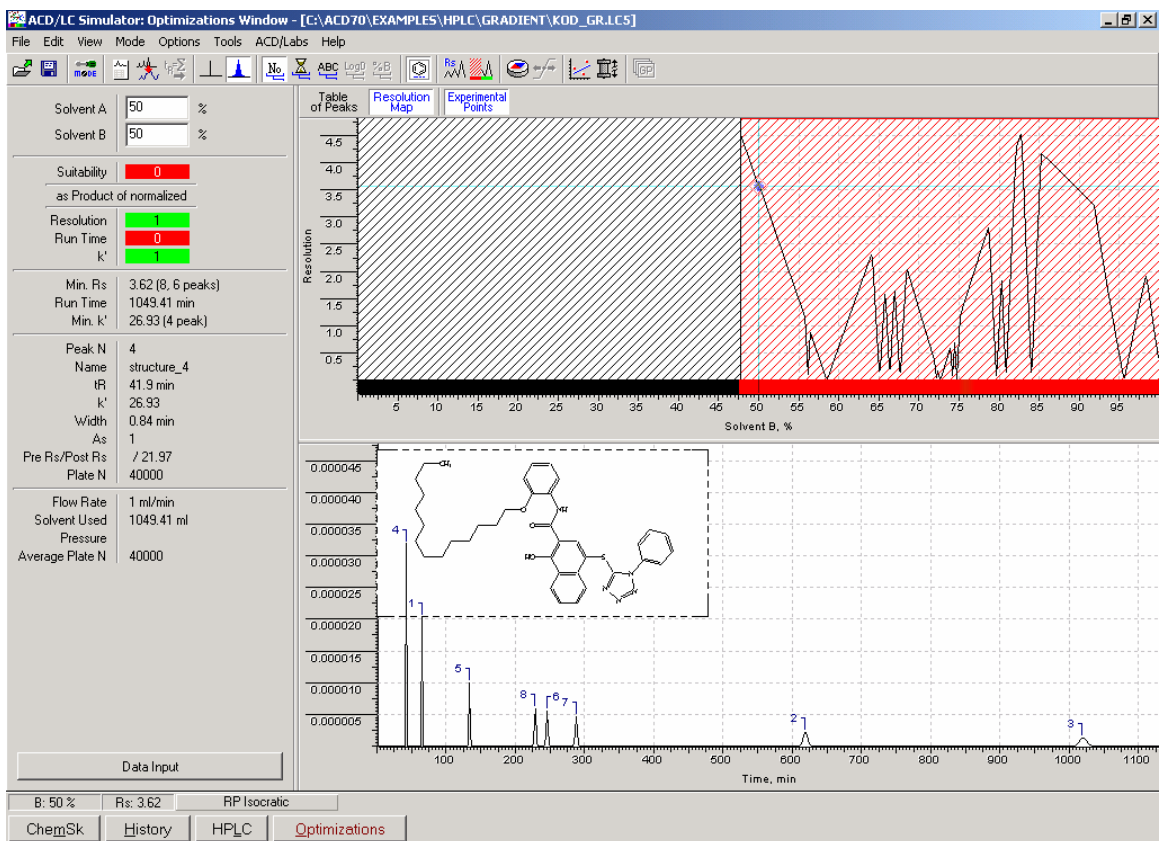


Q8. Does this offer any improvement in the chromatogram?

This procedure can be repeated which, for more complicated sample mixtures, can be used to tune the resolution for specific pairs of peaks. The file "Kod_gr_end.lc5" shows a slightly improved gradient option for this separation.

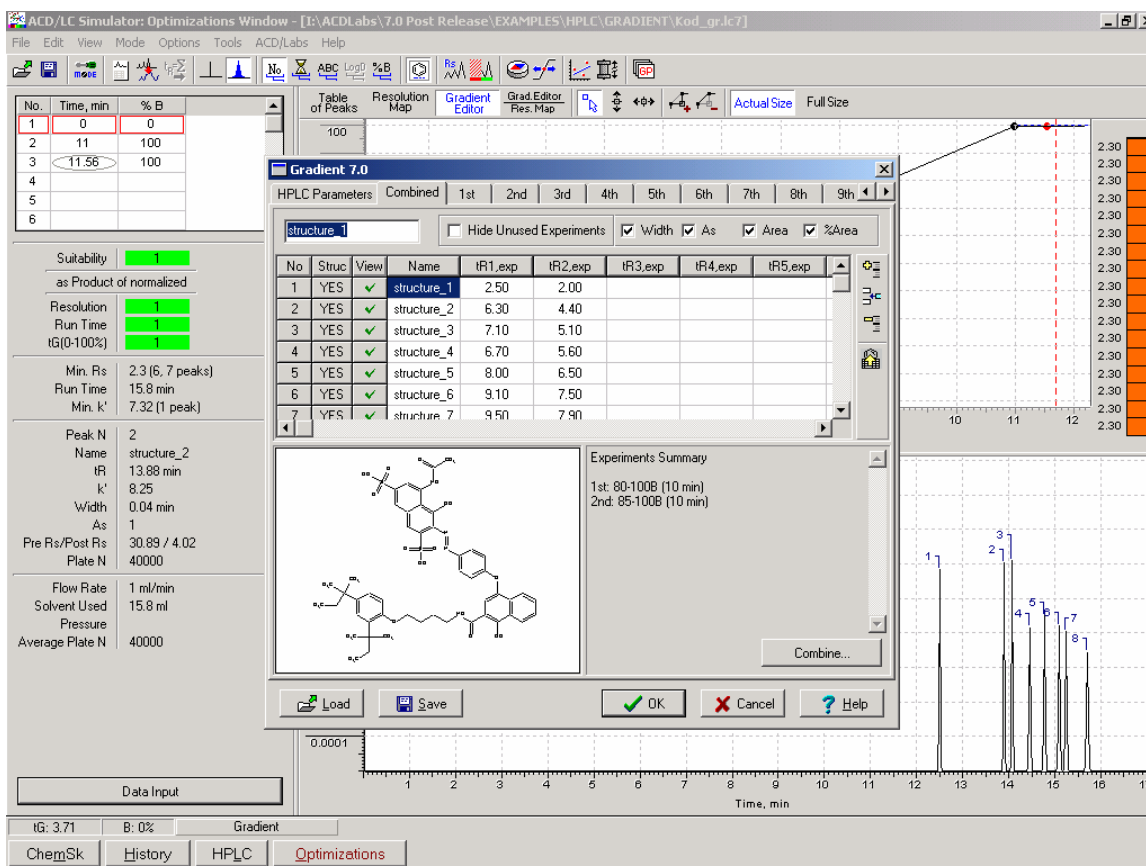
2.10 Simulate an isocratic separation. To do this, choose the RP isocratic mode. From the resulting resolution map, examine some of the better solutions to the problem.





Q9. What is the best isocratic solution? Why? What are the benefits of isocratic separations versus gradient methods?

2.11 Click **Data Input** and select one of the original two chromatograms. You can review the structures of the actual compounds by clicking on their name field (structure_1 etc.).



Q10. Looking at these structures, how do you explain the ability of the solvents used in the mobile phase to elute these compounds? (Hint: consider the functional groups and structural elements, and the Polarity Index (P') values for the solvent used).

Bonus work

2.12 Using the same file in RP Isocratic Mode, vary the solvent strength and observe the movement of the peaks. In Excel, plot the $\log k'$ versus solvent strength curves for each of the components, varying the solvent strength in reasonable increments.

Q11. How is this plot related to the isocratic resolution map?

2.13 There are some systems on which reasonable separations cannot be achieved without using gradients. Sometimes it is necessary to use stepwise gradients to “stretch and contract” various parts of the chromatogram. To look at a more difficult separation, open the file "LC_grad_AAA.lc7". Once again, the training set is two gradient separations. Switch to RP Isocratic Mode and observe that no isocratic solution exists. Switch back to gradient mode and design a method that gives a reasonable separation. Hint: work left to right, adding points to the gradient and optimizing each point individually. You may want to optimize a given point using the “auto-optimization” command.

Q12. Select a peak near the end of the chromatographic run. Add a point to the gradient just before the elution time of the compound and introduce a steep gradient after this point. What happens to the retention time of this compound? Why?