

Translation of Gradient Methods to Isocratic Methods Within ACD/LC Simulator

Version 8.0

Irina Oshchepkova and Mike McBrien
Advanced Chemistry Development, Inc.
Toronto, ON, Canada
www.acdlabs.com

With the help of this Technical Note we will demonstrate how to switch between Gradient and Isocratic Modes in order to use two or more gradient experiments to model the best isocratic solution to a method development problem.


Isocratic chromatographic experiments have several advantages over gradient runs, particularly for samples that will be run on a routine basis:

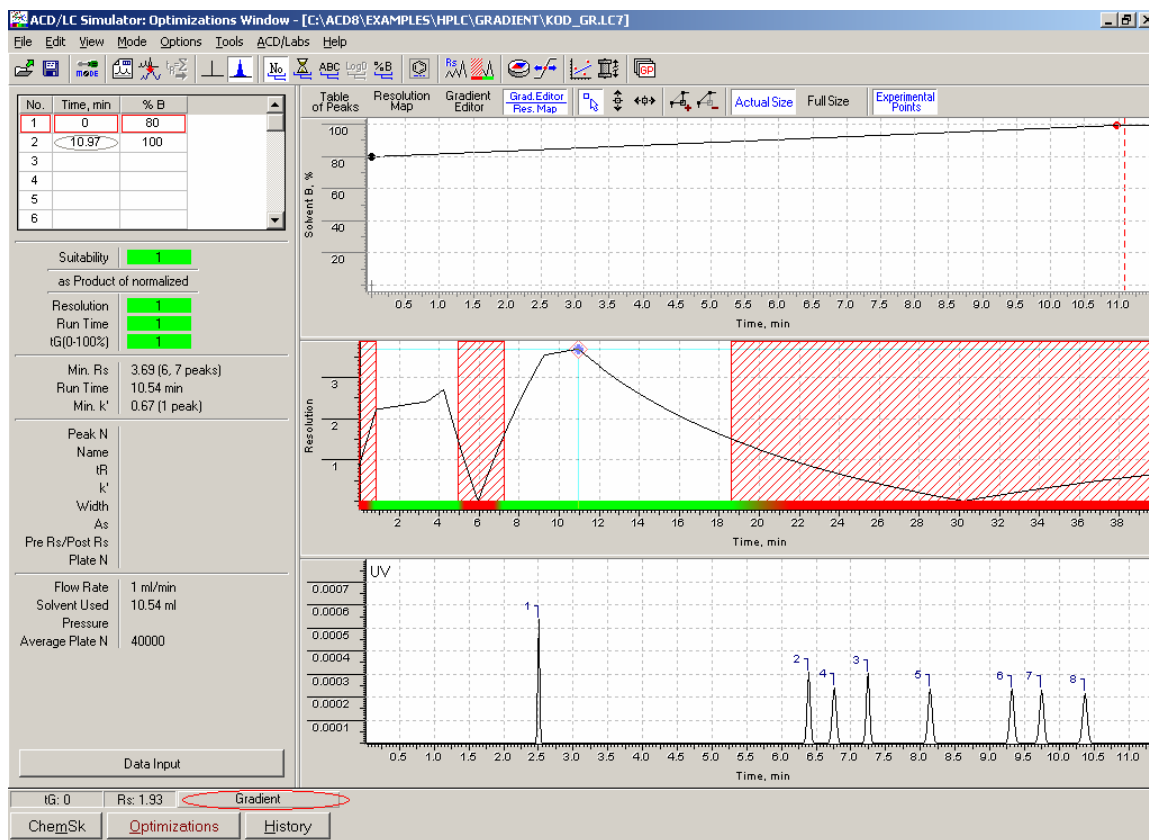
1. Reproducibility—the added complexity of the gradient system can lead to issues with the reproducibility of conditions.
2. Equilibration—it takes time to return to starting conditions and equilibrate prior to the next analysis.
3. Transferability—differences in dwell volumes between systems lead to issues with gradient transfer from instrument to instrument.
4. Simplicity of instrumentation—in some cases, analysis labs may not have gradient-capability available in all instruments.

In [ACD/LC Simulator](#) [1], the chromatographer can perform two or more scouting gradients and then determine if an isocratic solution is likely to be possible in just a few seconds. For separations in which an isocratic method is nonviable, automated tools for gradient generation are described.

Isocratic Possible

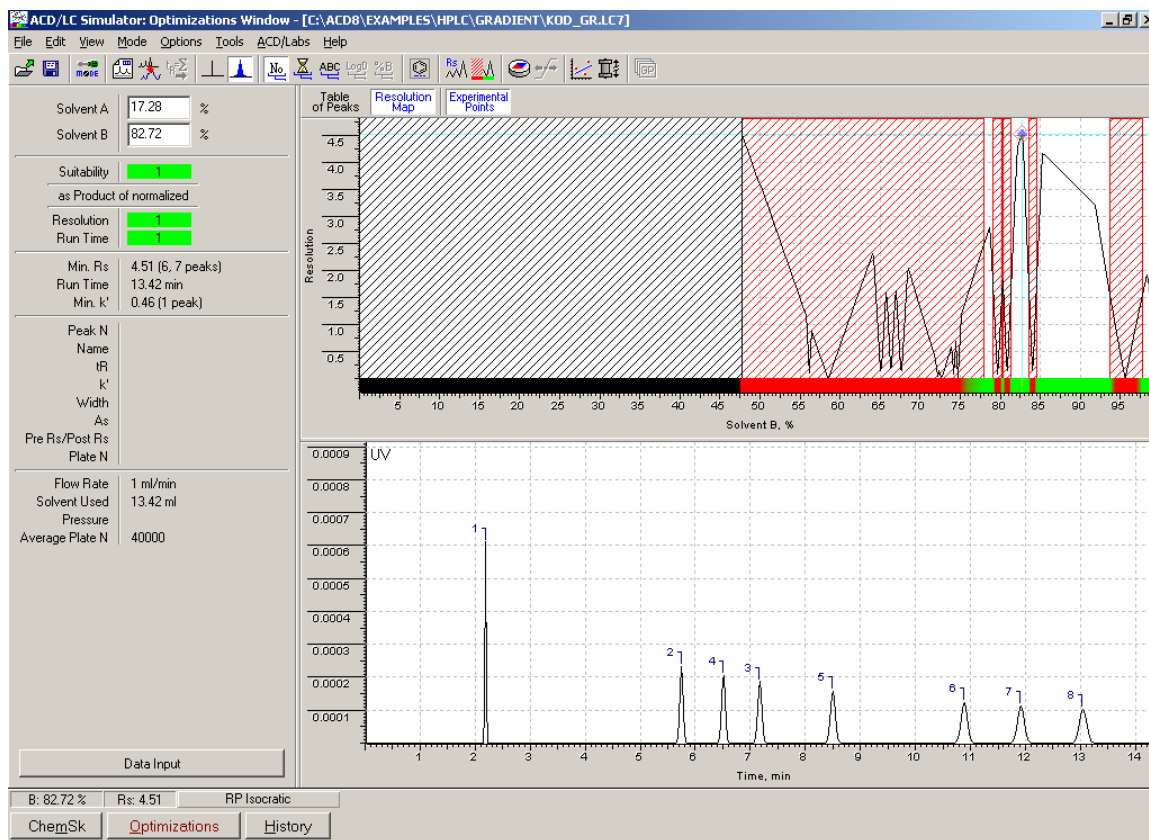
Note All the examples are shown with the existing *.LC7 files. To create this file, the user can open experimental data in [ACD/ChromManager](#) [2], transfer them to ACD/LC Simulator and save in *.LC7 format for further optimization.


1. Open ACD/LC Simulator, the Optimization window.
2. Open the *KOD_GR.LC7* file from the \EXAMPLES\HPLC\GRADIENT folder.
3. Search optimal conditions by clicking **Search Optimal Conditions** .
4. You should see the following:




- The button on the bottom that tells you what mode is applied to the chromatograms. In this case it is **Gradient** linear; two gradient experiments were used to train the system.
- From the **Mode** menu, choose **RP Isocratic**. You will see how your experiment will look in Isocratic mode.

Note Please note that we had a linear model in the Gradient Mode and switching to RP Isocratic as described above will not change the linear dependence.

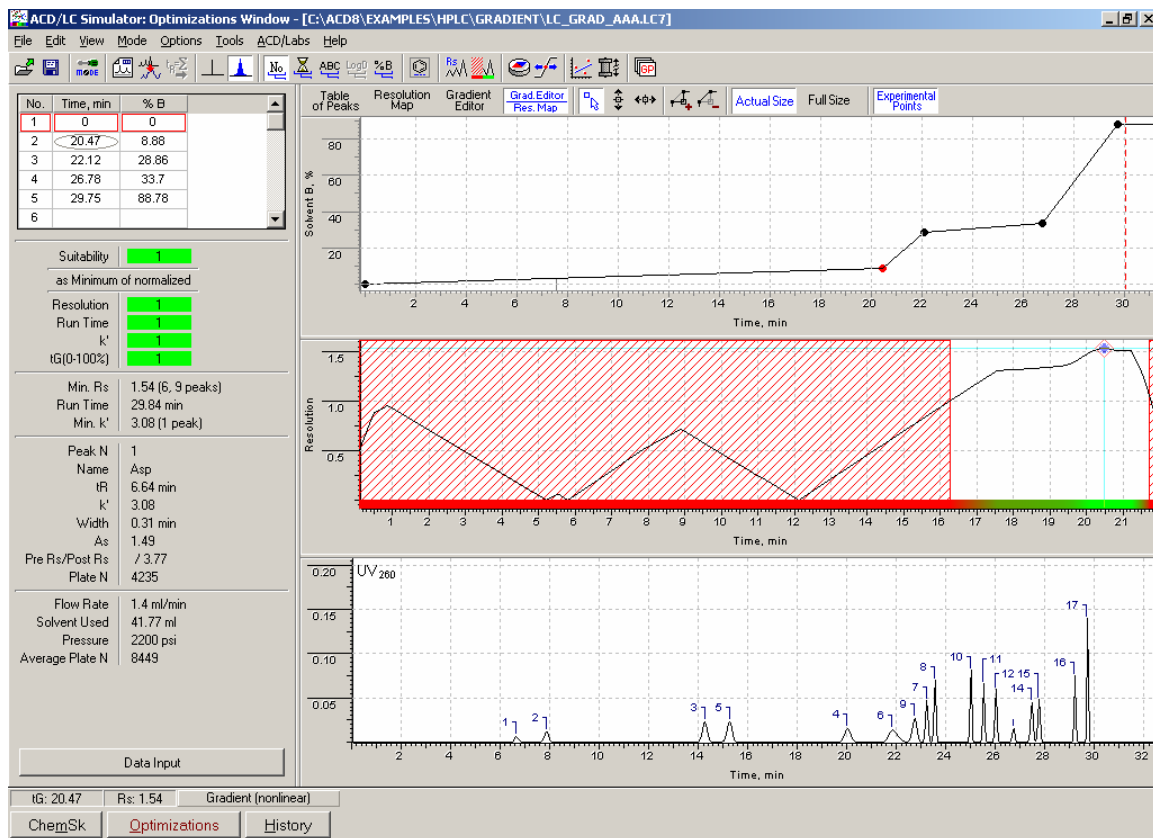


7. Click **Search Optimal Conditions**  to see the optimal conditions.
8. The result here is the best isocratic separation based on the suitability settings that were input. The next step would be to try this method, and confirm the accuracy of the prediction. Where necessary, the system could be retrained to increase the accuracy of the model, and re-optimized.
9. If no reasonable solution is found, then a gradient can be designed (see below).

Gradient Solution Required

1. Open ACD/LC Simulator, the Optimization window.
2. Open the *LC_GRAD_AAA.LC7* file from the \EXAMPLES\HPLC\GRADIENT folder.
3. Search optimal conditions by clicking **Search Optimal Conditions** .

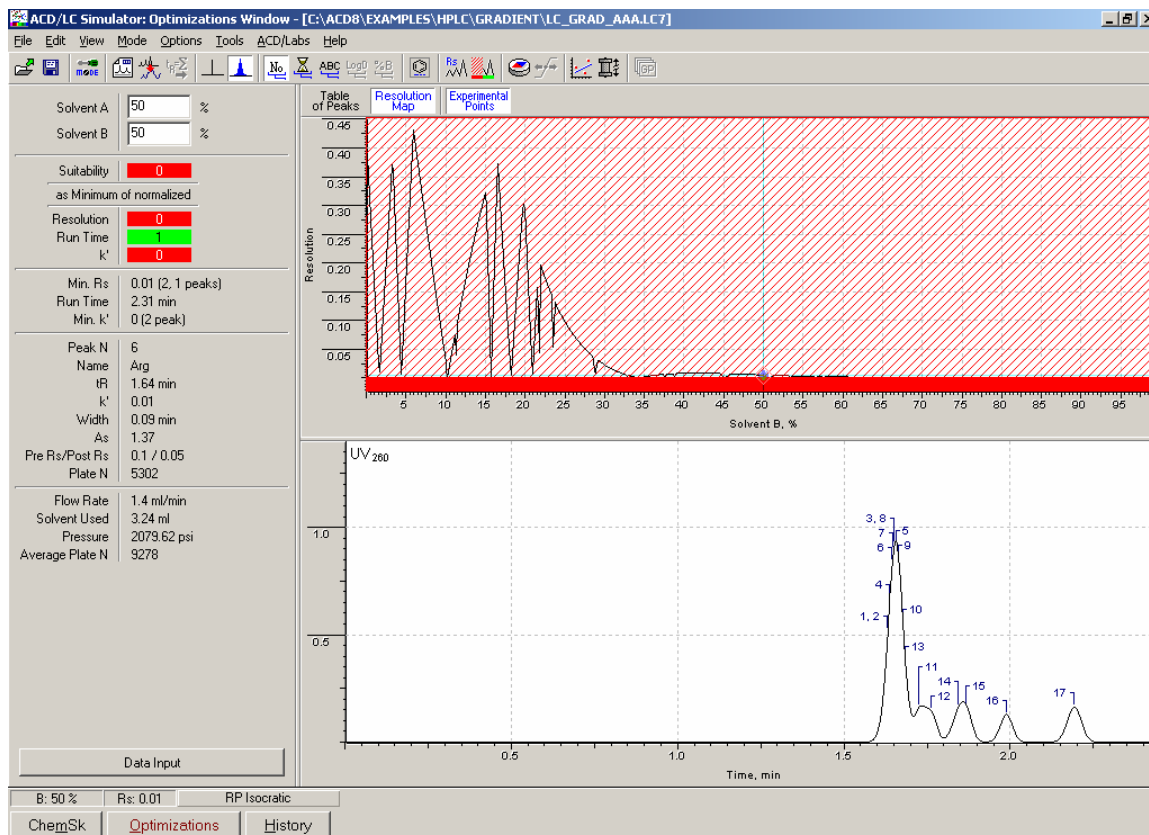
4. You should see the following:



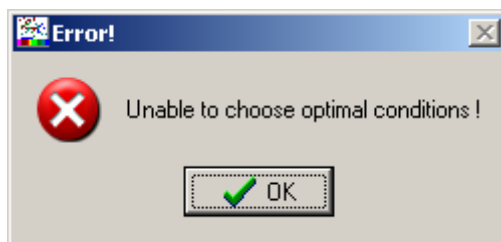
- Notice the button on the bottom that tells you what mode is applied to the chromatograms. In this case it is **Gradient (nonlinear)**
- From the **Mode** menu, choose **RP Isocratic**. You will see how your experiment will look in Isocratic mode. You can also click **Gradient (nonlinear)** and when the list with available modes appears, select **RP Isocratic**.

Note Using this method for switching to Isocratic Mode will change your non-linear model to linear.

7. You will see a lot of co-eluting peaks:

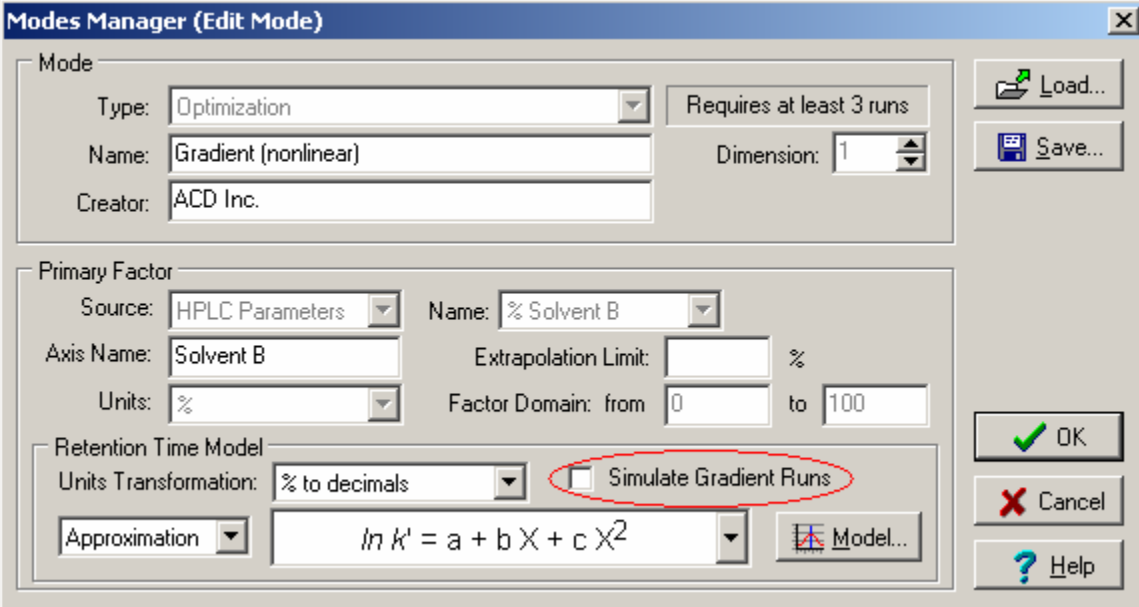


8. Notice that clicking **Search Optimal Conditions**  will give you an error:



9. In this case, an isocratic solution is not available. The user can switch back to gradient mode and use various tools in order to design an effective separation here. Note that for relatively simple gradients, one-step gradient generation and Gradient Generator are excellent tools.

10. If the user wants to take a look at the data in Isocratic Mode without changing the non-linear model, he/she should do the following: from the **Mode** menu, choose **Modes Manager**, and in the dialog box that appears, click **Edit** and click to clear the **Simulate Gradient Runs** check box.



Modes Manager (Edit Mode)

Mode
 Type: Optimization
 Name: Gradient (nonlinear)
 Creator: ACD Inc.

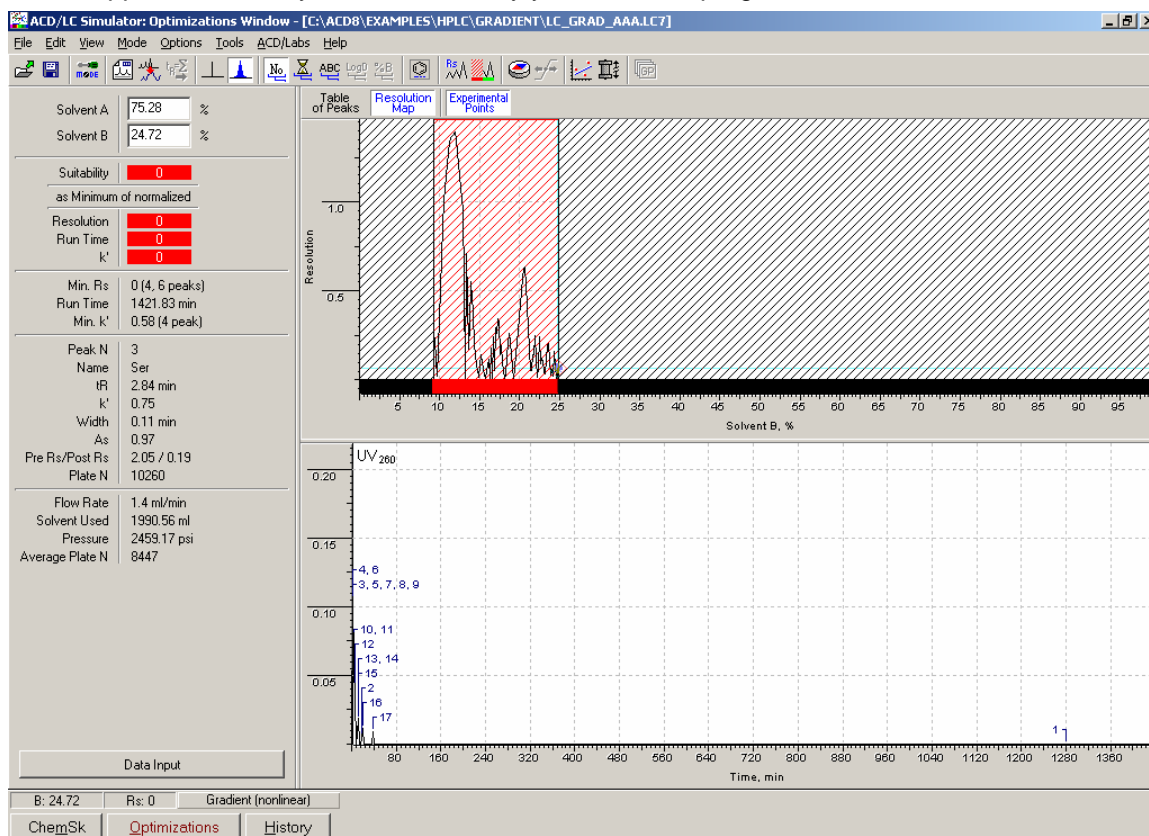
Requires at least 3 runs
 Dimension: 1

Primary Factor
 Source: HPLC Parameters
 Name: % Solvent B
 Axis Name: Solvent B
 Units: %
 Factor Domain: from 0 to 100
 Extrapolation Limit: %

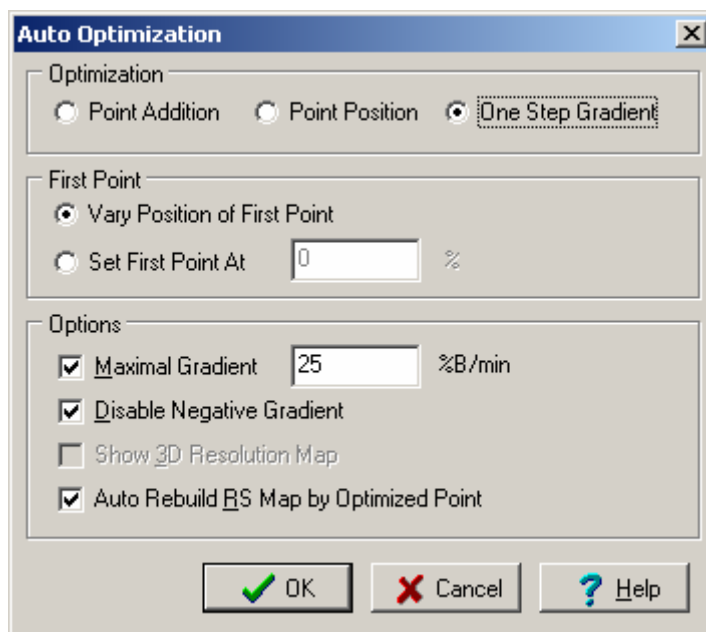
Retention Time Model
 Units Transformation: % to decimals
 Simulate Gradient Runs
 Approximation: $\ln k' = a + bX + cX^2$

Buttons: Load..., Save..., OK, Cancel, Help

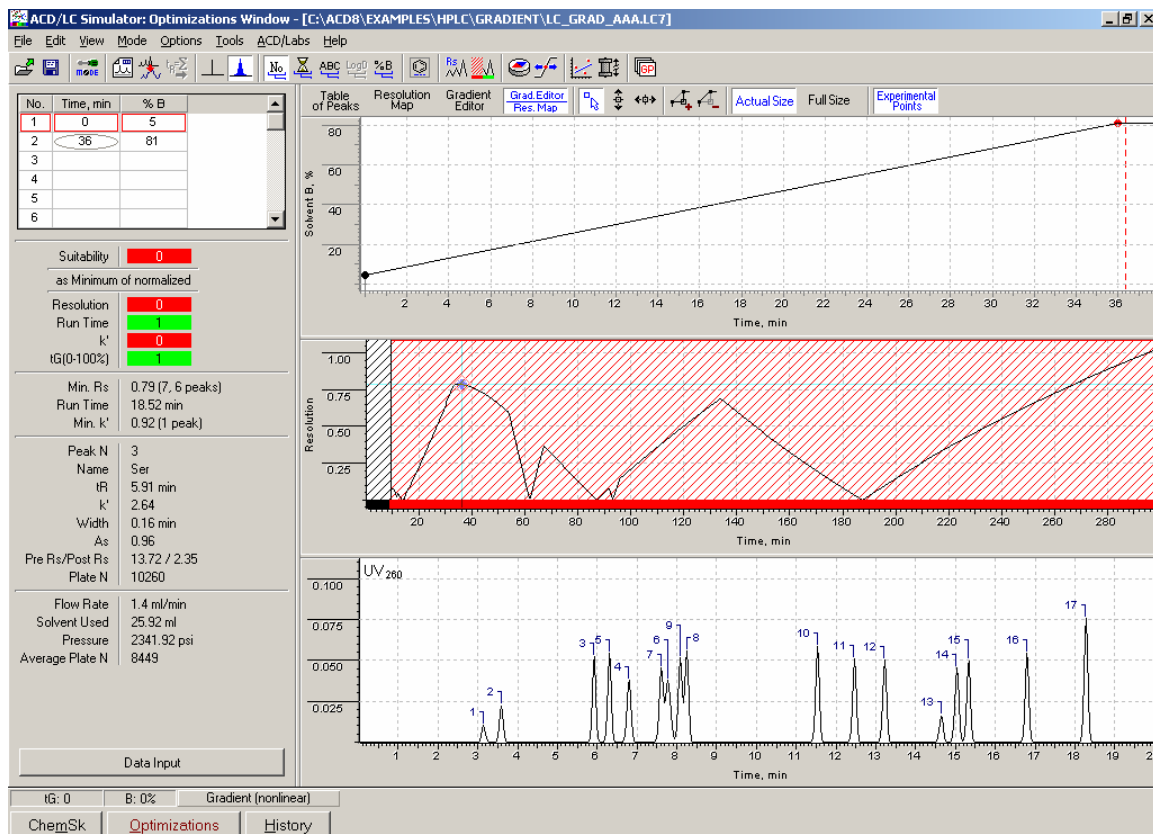
11. Then click **OK** two times to apply changes. You will see that the Isocratic method cannot be applied here and you need to modify your Gradient program.



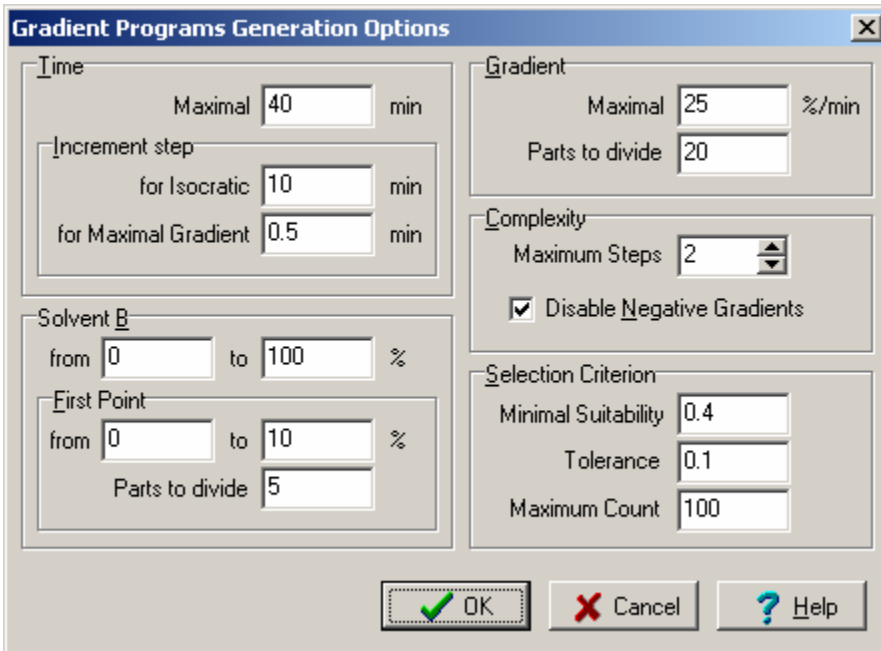
- Go back to Gradient (non-linear) mode by selecting the **Simulate Gradient Runs** check box as described in step 10.
- For the above sample, gradient auto-optimization (one step gradient) gives a reasonable separation. To do that, from the **Tools** menu, choose **Auto Optimization** and select the following options:



- Click **OK** and you will see the following:



15. In comparison, the Gradient Generator gives a very good result when set to determine the best two-step gradient. From the **Tools** menu, choose **Generate Gradient Programs** and specify the following options:



Gradient Programs Generation Options

Time
Maximal: 40 min
Increment step:
for Isocratic: 10 min
for Maximal Gradient: 0.5 min

Gradient
Maximal: 25 %/min
Parts to divide: 20

Complexity
Maximum Steps: 2
 Disable Negative Gradients

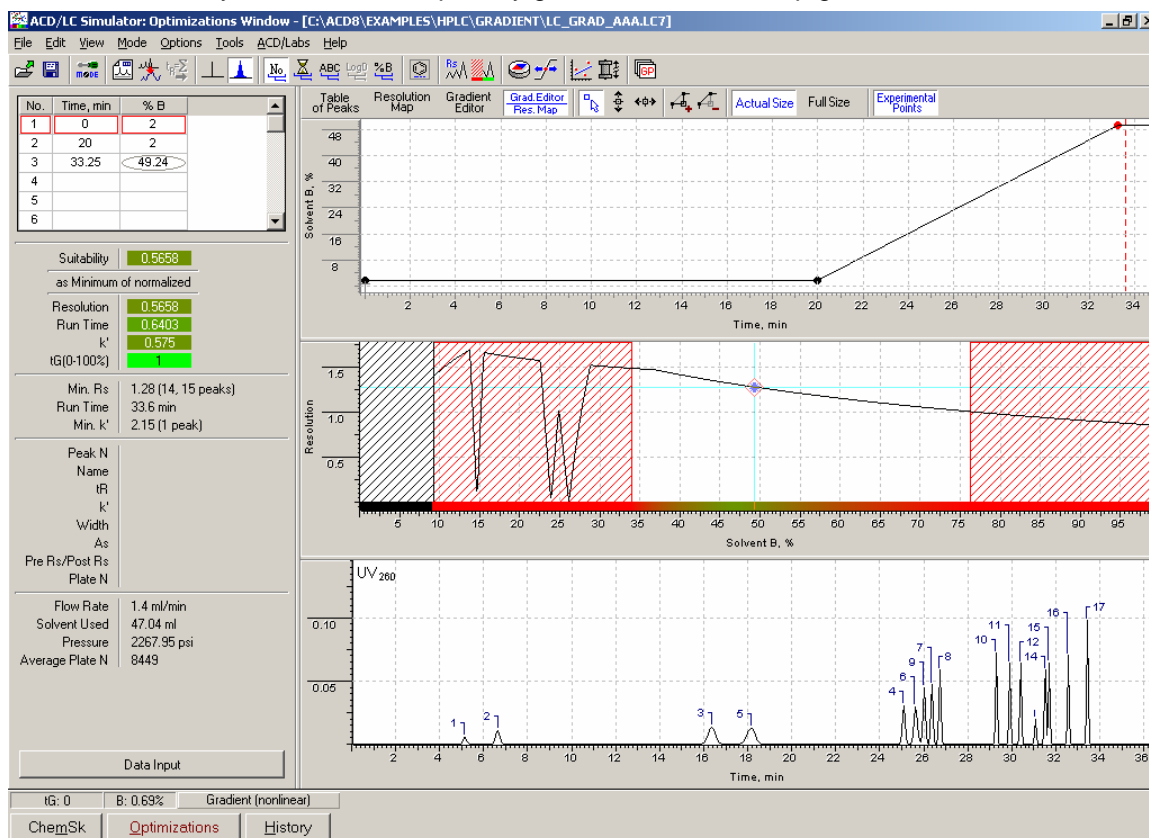
Solvent B
from: 0 to 100 %

First Point
from: 0 to 10 %
Parts to divide: 5

Selection Criterion
Minimal Suitability: 0.4
Tolerance: 0.1
Maximum Count: 100

OK Cancel Help

16. Click **OK** and you will see a comparably good result for two step gradient:



Conclusion

In ACD/LC Simulator, there are a number of tools for the optimization of mobile phase organic content. In this technical note, we saw how the chromatographer can use the versatility of gradient experiments to quickly investigate the elution profile of the sample, and then investigate the viability of an isocratic method. Where isocratic methods are not viable, automated gradient generation tools can then be used to design a simple or complex gradient according to the difficulty of the separation.

References

1. ACD/LC Simulator. <http://www.acdlabs.com/lcsim/>. January 3, 2005.
2. ACD/ChromManager. <http://www.acdlabs.com/chrommanager/>. January 3, 2005.