GC Method Development

Finding the Best Carrier Gas Average Linear Velocity:

Determining the best average linear velocity is fairly easy and only involves a small amount of trial and error. Hydrogen provides the best resolution in the shortest amount of time. Helium provides similar resolution, but at a longer analysis time. Nitrogen is not recommended for use with capillary columns due to the extremely long analysis times.

When using helium as the carrier gas, try an initial average linear velocity of 30 cm/sec. If better resolution is desired, reduce the velocity to no less than 25 cm/sec; however, the analysis time will be increased. If a shorter analysis time is desired, increase the velocity to 35 cm/sec and 40 cm/sec. Be aware of potential resolution losses at these higher linear velocities. Average linear velocities of 30-35 cm/sec are used for many analyses when using helium as a carrier gas.

When using hydrogen as the carrier gas, try an initial average linear velocity of 60 cm/sec. If better resolution is desired, reduce the velocity to no less than 50 cm/sec; however, the analysis time will be increased. If a shorter analysis time is desired, increase the velocity to 70 cm/sec and 80 cm/sec. Be aware of potential resolution losses at these higher linear velocities. Average linear velocities of 60-70 cm/sec are used for many analyses when using hydrogen as a carrier gas.

Upon comparing the chromatograms at the various average linear velocities, retention and resolution differences will be noticeable. Sometimes different average linear velocities are best for different peaks within the same chromatogram. In these cases, a compromise velocity is usual selected. Except with nitrogen, small changes in the average linear velocity (<2 cm/sec) rarely result in significant changes in resolution. When experimenting with average linear velocities, try values that are different by at least 3-4 cm/sec.

Default Injector Settings :

Most samples can be analyzed using a wide range of injector conditions or parameters. This results in a fairly standard set of injector conditions being suitable for most samples. Since the default or standard injector conditions are suitable for 80-90% of all samples, these conditions are a good place to start when developing a new method.

An injector temperature of 250?C is sufficient for nearly all samples. For volatile samples such as volatile solvents, an injector temperature of 150-200?C is recommended. For high boiling samples such as steroids, triglycerides or surfactants, an injector temperature of 275-300?C is recommended. Make sure the septum can tolerate the high injector temperature.

Megabore Direct

Temperature: 250?C
Liner: Direct flash vaporization
Injection volume: 1 ?L

Split

Temperature: 250?C
Liner: Straight tube or hourglass shape
Injection volume: 1 ?L
Split ratio: 1:50

Splitless

Temperature: 250?C
Liner: Straight tube with a bottom restriction
Injection volume: 1 ?L
Purge activation time: 0.5 minutes

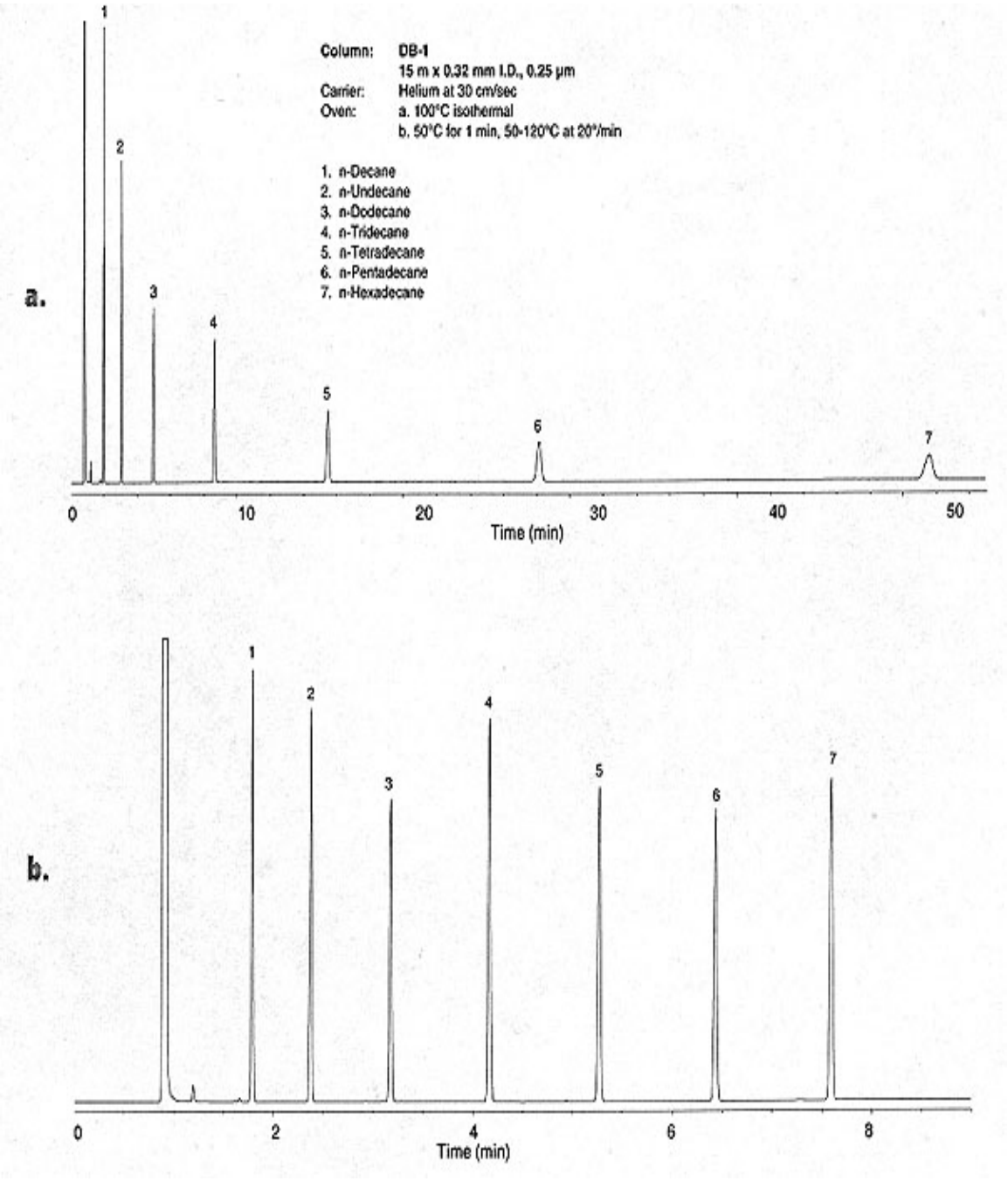
Oven Temperatures :

Isothermal temperature condition involves maintaining a constant oven temperature throughout the GC run. Isothermal temperature conditions are used for solutes with similar retention. Retention differences for dissimilar solutes can be quite severe for isothermal temperature conditions. Peak widths rapidly increase with retention for isothermal conditions (Figure 30a). For these reasons, isothermal temperature conditions are only suitable for a limited number of analyses.

Most analyses require the use of a temperature program. A temperature program involves heating the oven at a controlled rate during the run. This allows the faster analysis of solutes with dissimilar retention, and there is very little peak broadening with an increase in retention (Figure 30b). The primary disadvantages of a temperature program are the more difficult development process and the cool down time between analyses. There are no secrets or tricks to finding the best temperature program for an analysis. Usually some trial and error is involved.

If numerous attempts at different temperature programs has not resulted in satisfactory peak resolution, a different approach may be necessary. Some compounds cannot be separated with a particular stationary phase with any reasonable temperature program, thus a different stationary phase may be necessary. Sometimes, improving efficiency may be the answer. Optimizing the carrier gas average liner velocity, improving injector efficiency, or using a more efficient column dimension may provide the desired resolution.

Figure 30. Isothermal vs. Temperature Program Conditions.



A Warning When Adjusting Temperature Programs :

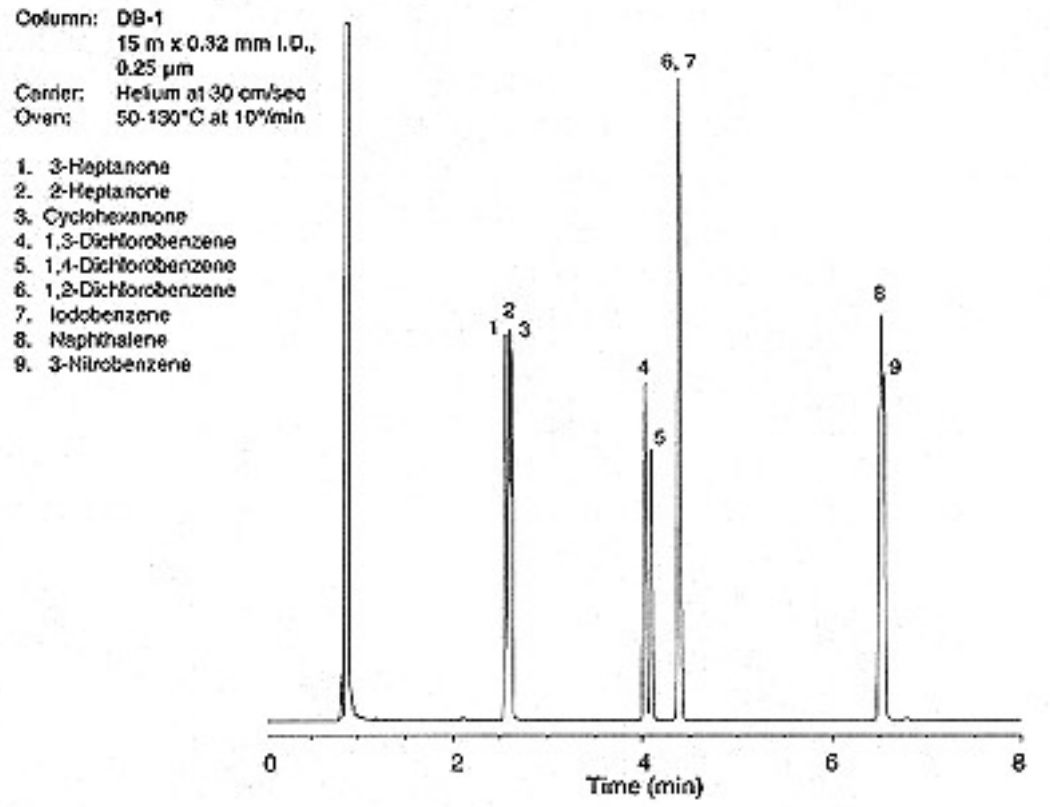
When changing a temperature program, confirmation of peak identities in the new chromatogram is essential. Peak retention orders can shift upon a change in the temperature program (called peak inversions). Peak misidentifications or an

apparent loss of a peak (actually co-eluting with another peak) are common results of undetected peak inversions.

Developing a Temperature Program :

Using a Linear Temperature Program as a Starting Point
If previous analysis information is not available to use as a guide, the first program development step is to try a simple, linear temperature program. This provides information on the retention characteristics of the solutes. Start with an initial temperature of 50?C, a ramp rate of 10?/min, a final temperature equal to the isothermal temperature limit of the column, and a final hold time of approximately 30 minutes. The long final hold time is used to ensure all of the solutes elute from the column. The program can be stopped several minutes after the last solute has eluted from the column. This may occur before the final temperature is reached (Figure 31). After obtaining a chromatogram using the simple, linear temperature program, the next steps are to adjust the various program components to obtain adequate resolution and the shortest analysis time.

Figure 31. Simple, Linear Temperature Program



Adjusting the Initial Temperature and Hold Time :

To improve the resolution of earlier eluting peaks, decrease the initial temperature or increase the initial hold time. Decreasing the initial temperature usually results in the largest resolution improvement, but analysis times are substantially increased (Figure 32a). In addition, cool down times between runs can be significantly increased especially when cooling below 50?C.

The resolution of the later eluting peaks are minimally affected by lowering the initial temperature especially for longer length columns. If excessive resolution is obtained with the original linear temperature program, increase the initial temperature to reduce the resolution and analysis time. The resolution of later eluting peaks may also be reduced upon increasing the initial temperature.

Increasing the initial hold time often improves the resolution of the earlier eluting peaks; however, the improvement is smaller than those obtained with lowering the initial temperature (Figures 32b and c). The resolution of later eluting peaks is minimally affected with a change in the initial hold time. Lowering the initial temperature and increasing the initial hold time can be combined to improve the resolution of earlier eluting peaks (Figure 32d). Hold times should be limited to 5 minutes or less if possible. Peaks eluting during the later portion of the hold time may start to broaden, thus making resolution more difficult to achieve.

Adjusting the Ramp Rate :

The resolution of the peaks eluting in the middle of the chromatogram can be altered by changing the ramp rate. If there is excessive peak resolution, the ramp rate can be increased to reduce the resolution and the analysis time. If there is insufficient resolution, decrease the ramp rate, but there will be an increase in the analysis time (Figure 33a). Better resolution of later eluting peaks often occurs when decreasing the ramp rate. Only change the ramp rate by about 5?/min each time. Much larger or smaller alterations usually cause massive or insignificant changes, respectively. Changes in initial temperatures and times can be combined with ramp rate changes to affect a large section of the chromatogram (Figures 33b-d).

Multiple ramp rates can be used to affect smaller regions of the chromatogram. For example, if 5?/min was good for the earlier portion of the chromatogram and 15?/min was better for a later portion, both ramp rates can be used within a single program (Figure 34).

Figure 32. Changing Initial Temperatures and Hold Times.

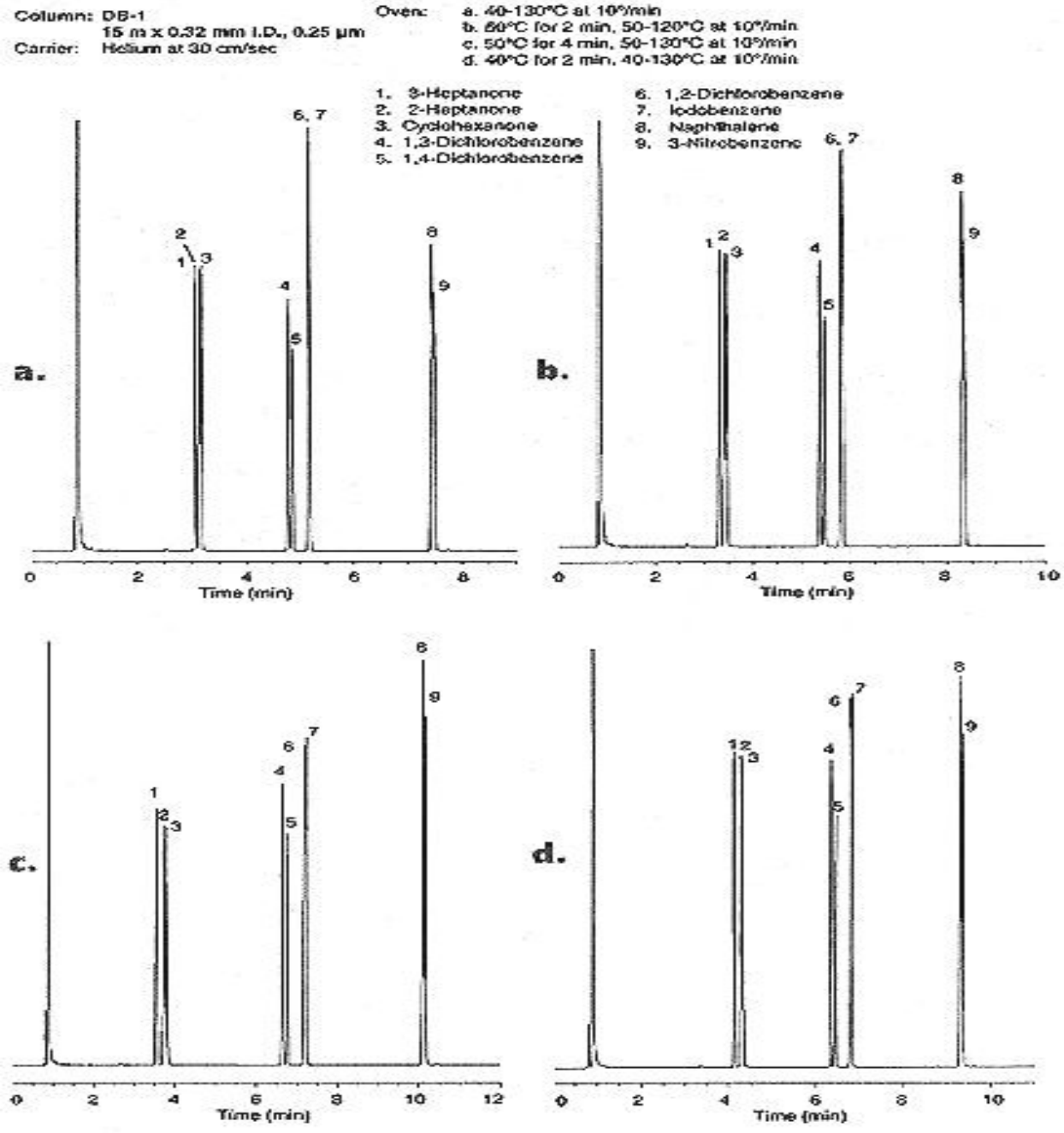


Figure 33. Changing the Ramp Rate

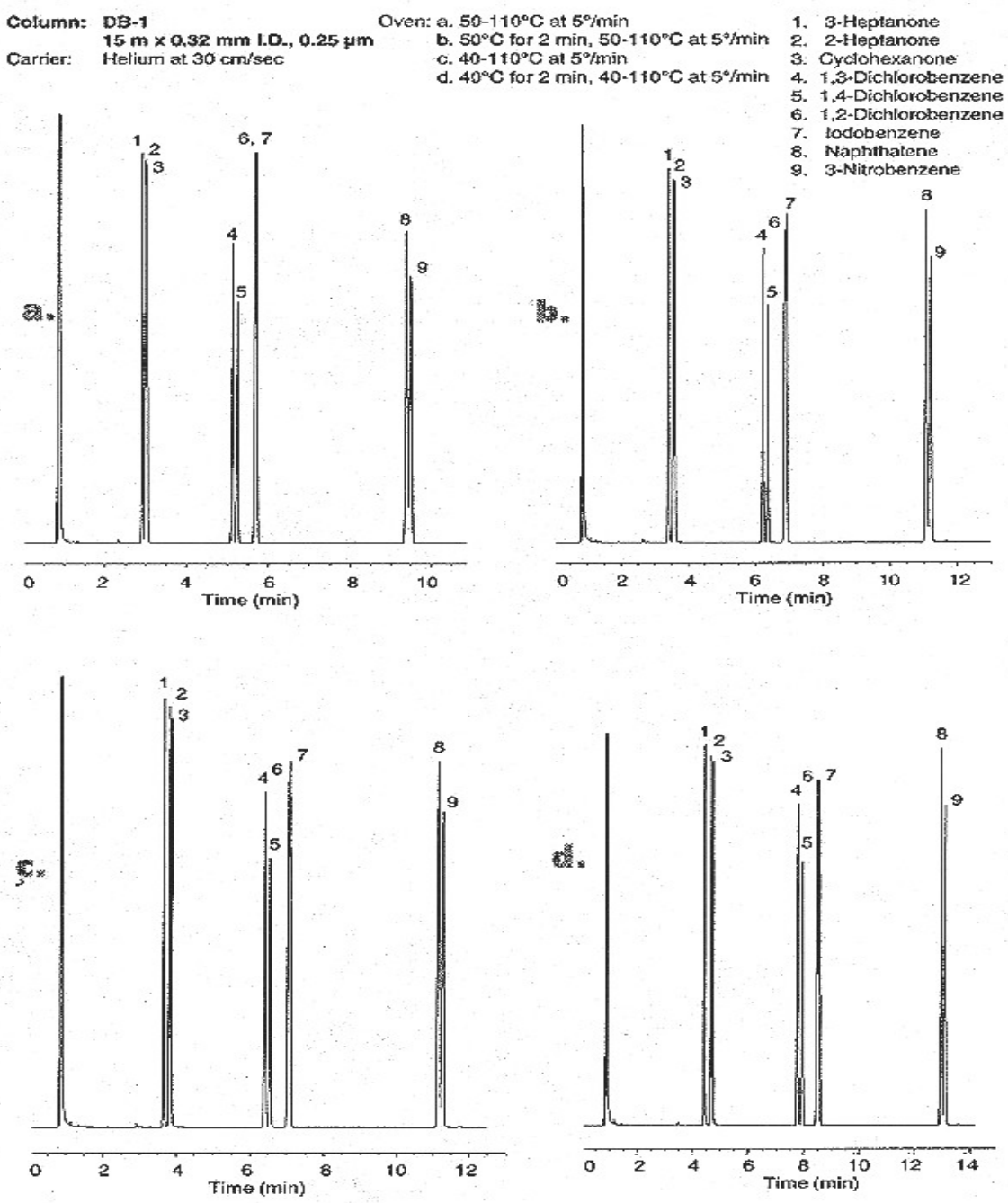
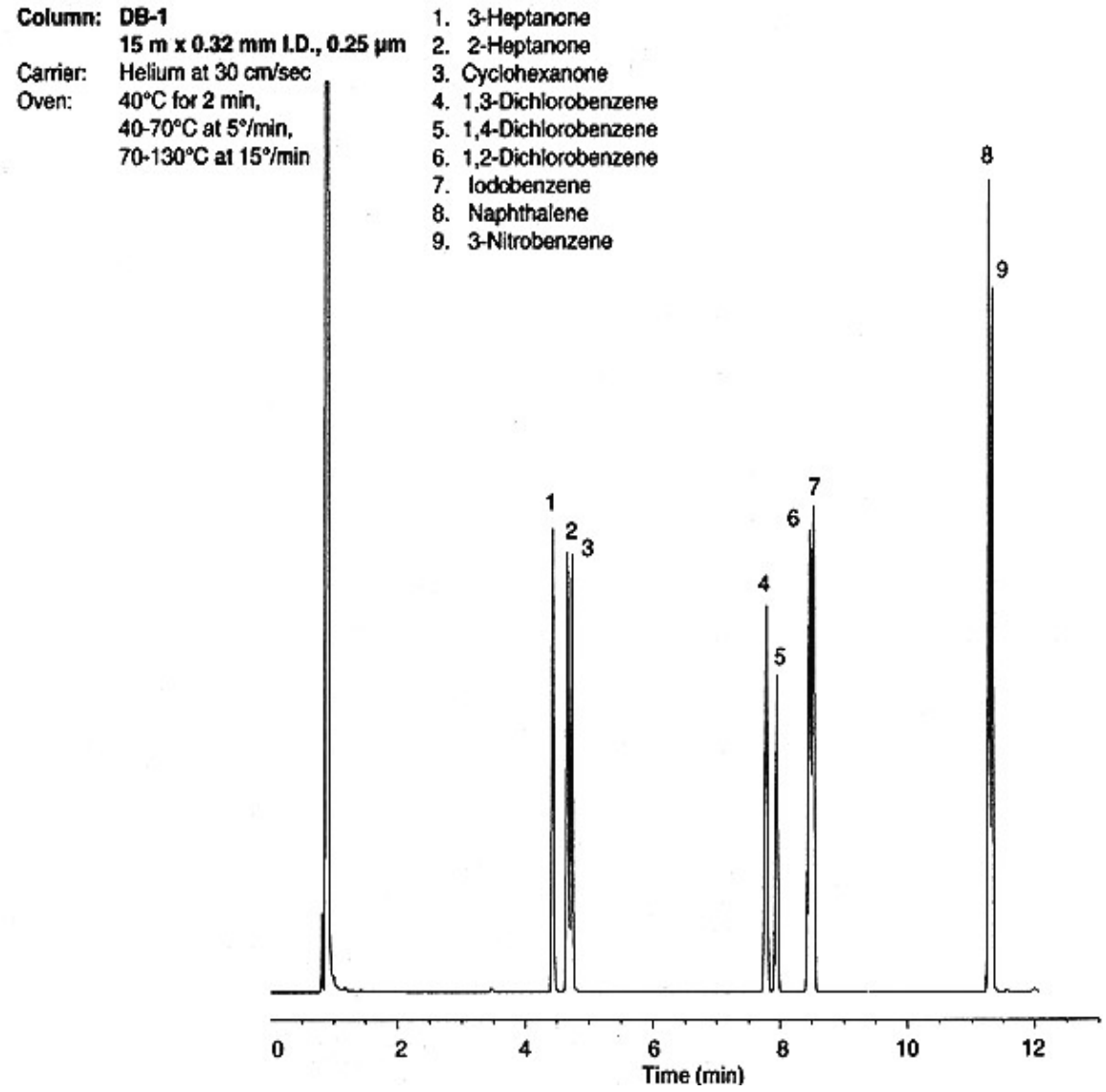
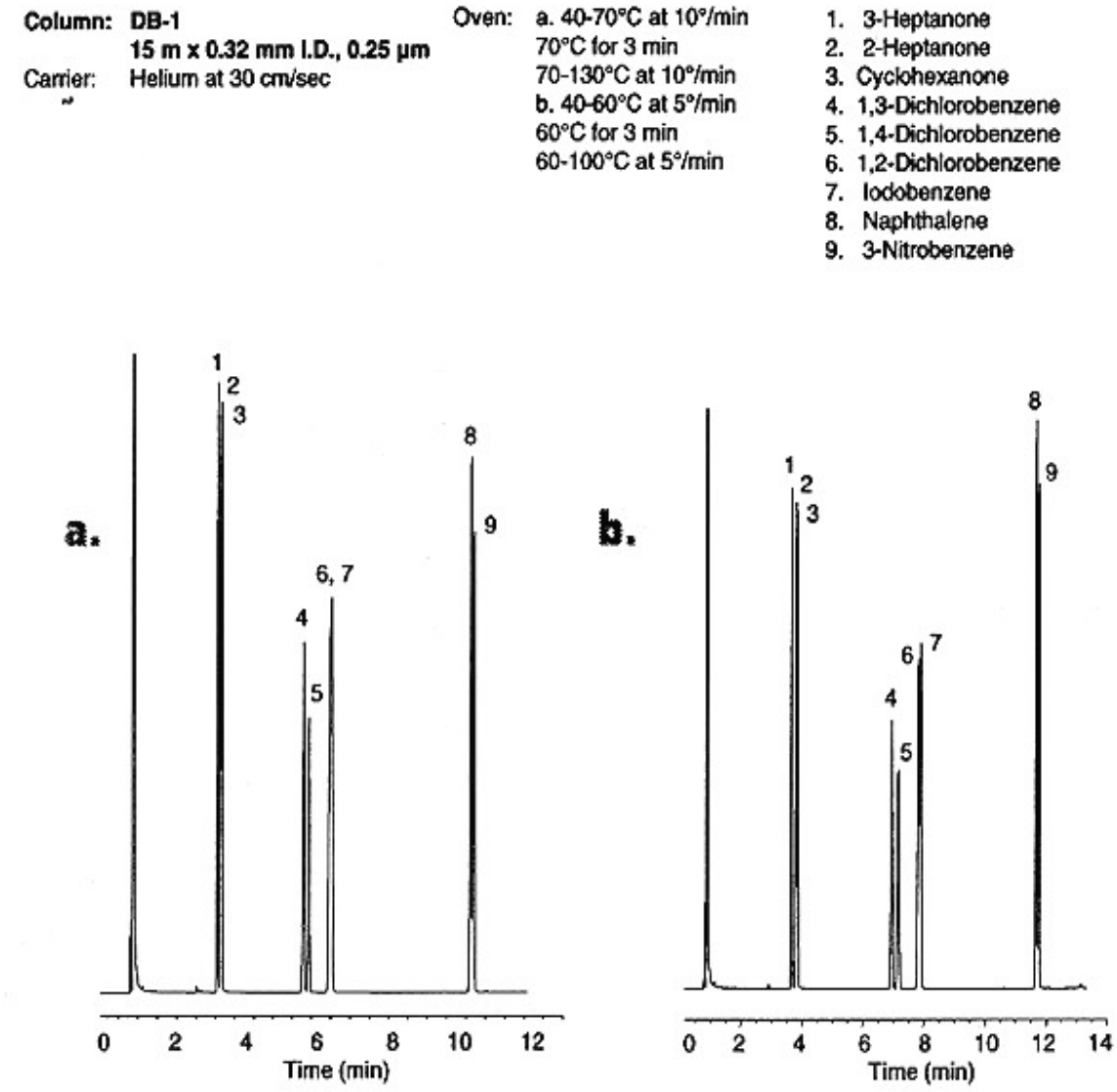


Figure 34. Using Multiple Ramp Rates



Another option to alter resolution of peaks in the middle of a chromatogram is to use a mid ramp hold. A mid ramp hold is a several minute isothermal portion somewhere during a temperature ramp. For example, the temperature program of 50-100?C at 10?/min, 100?C for 3 min, 100-300?C at 10?/min contains a mid ramp hold. To determine a suitable hold temperature, calculate the oven temperature range when the first peak of interest is eluting. Use a hold temperature that is 20-30?C below this temperature. Hold times of 2-5 minutes are most effective. Shorter or longer times often have no, or a detrimental, affect on peak resolution. Try several different temperatures and hold times since small changes in the times and temperatures can be significant (Figures 35a and b). Use a mid ramp hold only if other temperature program changes were not effective.

Figure 35. Using Mid Ramp Holds



Final Temperature and Time :

Stop the temperature program shortly after the last peak has eluted from the column. If the column's isothermal temperature limit is reached and peaks are still eluting, a final hold time is necessary. Only use a final hold time if the temperature limit is reached. Any peaks that elute during isothermal temperature conditions substantially increase in width as peak retention increases.

Extracted samples often contain compounds that elute after the last solute of interest. The final temperature and/or hold time need to be large enough to ensure elution of these compounds. Higher final temperatures or longer hold times should be tried until it is certain that all solutes elute from the column for every run. Column contamination will occur if portions of previously injected samples remain in the column during later injections.

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