Gas Chromatography Problem Solving and Troubleshooting

Question:

In an attempt to lower the amount of column bleed and decrease the run time for a drug analysis, a 0.10-µm film column was used instead of a 0.30-µm film column. Many of the peaks exhibited poor shapes and decreased resolution with the 0.10-µm column. Changing the temperature program or carrier gas velocity did not improve the situation. Why did the peak shapes deteriorate, and can satisfactory peaks be obtained with the 0.10-µm film column?

Answer:

Whenever a column dimension is changed, multiple performance characteristics are usually simultaneously affected. Some of the chromatographic changes may be relatively insignificant or unimportant, whereas others are quite prominent, with some being undesirable. Capillary column film thickness directly affects a number of parameters including retention, column bleed, sample capacity, activity, and efficiency. Upon changing to the 0.10-µm film column, not only was the retention and column bleed decreased, an accompanying increase in activity and decrease in capacity occurred. Although all of these factors may not have influenced the results, they all have to be considered. Most likely, a combination of these factors is responsible for the peak shape and separation problems experienced with the 0.10-µm column.

Column activity is primarily caused by compound interaction with silanols on the surface of the column's fused-silica tubing. Activity is visible as tailing peaks for compounds containing functional groups that readily undergo hydrogen bonding, such as hydroxyls and amines. In severe cases, significant loss of peak size also occurs. Column activity tends to increase as film thickness is decreased. Peak tailing is not obvious in the chromatograms for the 0.30- and 0.10-µm film columns (Figures 1A and 1B). The lack of peak tailing strongly indicates that the potential higher activity of the 0.10-µm film column is not

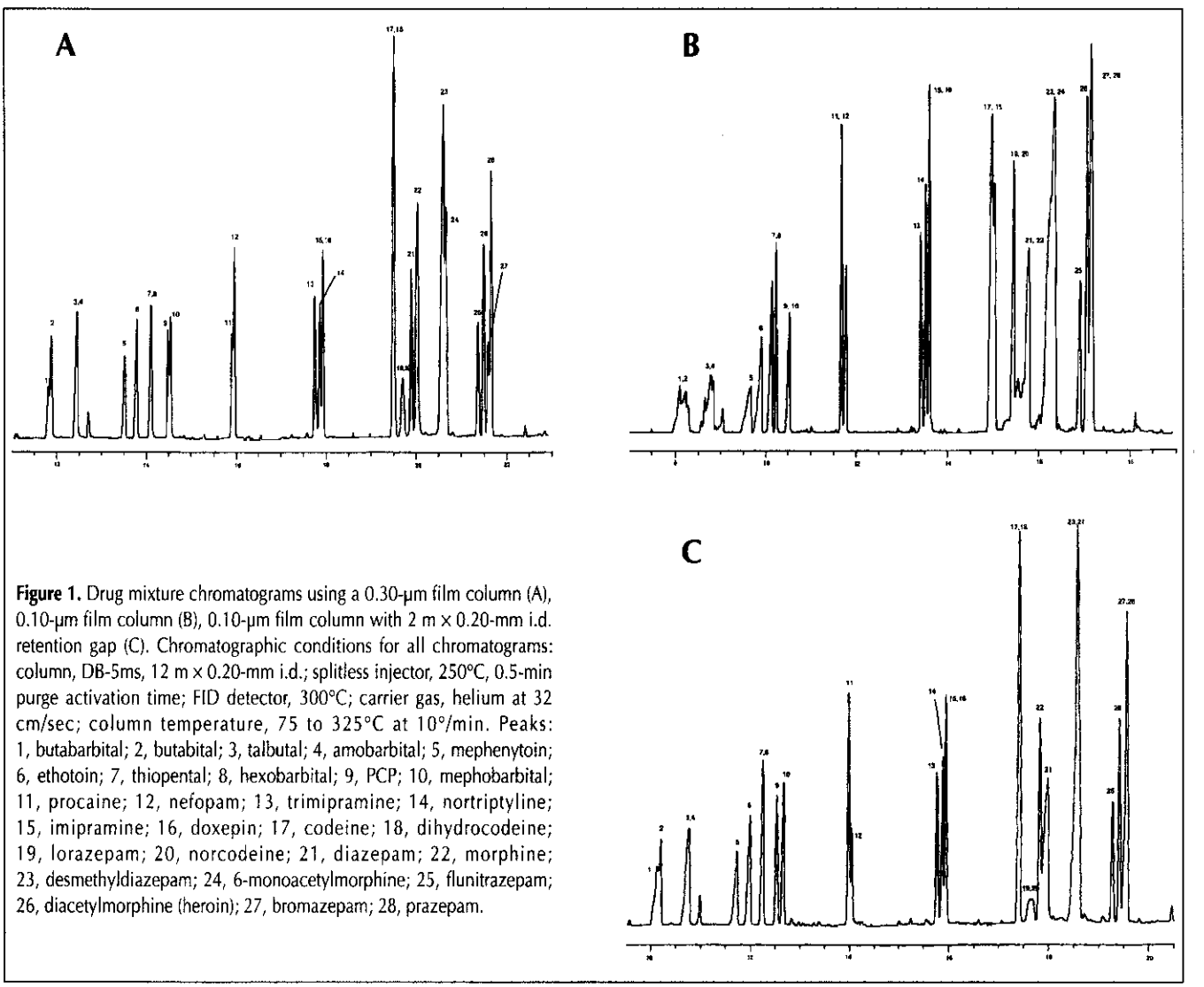

Figure 1. Drug mixture chromatograms using a 0.30-µm film column (A), 0.10-µm film column (B), 0.10-µm film column with 2 m x 0.20-mm i.d. retention gap (C). Chromatographic conditions for all chromatograms: column, DB-5ms, 12 m x 0.20-mm i.d.; splitless injector, 250°C, 0.5-min purge activation time; FID detector, 300°C; carrier gas, helium at 32 cm/sec; column temperature, 75 to 325°C at 10°/min. Peaks: 1, butabarbital; 2, butabital; 3, talbutal; 4, amobarbital; 5, mephenytoin; 6, ethotoin; 7, thiopental; 8, hexobarbital; 9, PCP; 10, mephobarbital; 11, procaine; 12, nefopam; 13, trimipramine; 14, nortriptyline; 15, imipramine; 16, doxepin; 17, codeine; 18, dihydrocodeine; 19, lorazepam; 20, norcodeine; 21, diazepam; 22, morphine; 23, desmethyldiazepam; 24, 6-monoacetylmorphine; 25, flunitrazepam; 26, diacetylmorphine (heroin); 27, bromazepam; 28, prazepam.

responsible for the loss of peak shape and separation.

Column capacity decreases as film thickness decreases. As the capacity of a column is exceeded, peak shapes become asymmetrical and broad. The front edge of an overloaded peak takes on a less steep slope than the back edge of the peak. An overloaded peak is often described as having a "shark fin" shape. Some of the earlier eluting peaks for the 0.10-µm column (Figure 1B) exhibit a small amount of this behavior. A number of the other peaks do not appear to be overloaded, even though all of the compound amounts are very similar. This result indicates that there may be other or additional causes of the problem.

For this analysis, splitless injection was used. Because most of the injected and vaporized sample enters the column, a large volume of solvent is introduced into the column. Splitless injections depend on the compound and/or solvent to focus at the front of the column in a tight band. A lack of sample band focusing often results in poor peak shapes, especially for the earlier eluting compounds. This type of effect is visible in Figure 1B but not in Figure 1A. The 0.30-µm film column is more retentive and has a higher sample capacity than the 0.10-µm film column. This results in better sample band focusing and satisfactory peak shapes for the 0.30-µm film column. The lack of sample focusing is the primary cause of the poor peak shapes. Because many of the peaks are poorly formed or broad, peak resolution is also reduced.

Sample band focusing problems are more prevalent with smaller diameter columns. The same volume of sample solvent occupies a longer length of column in smaller diameter columns. The longer sample band often translates into broad and misshaped peaks. Whenever sample band focusing problems occur, the use of a retention gap often improves or eliminates the poor peak shapes. A retention gap is usually 1–10 of deactivated fused-silica tubing attached to the front of the analytical column. Its diameter should be the same or larger than the analytical column. Figure 1C shows the resulting chromatogram when a 2-m retention gap is used with the 0.10-µm film column. Significant improvement is evident; however, the results are still not as good as with the 0.30-µm column without a retention gap.

Before a different column dimension is used for any analysis, it is important to understand the impact of the column change on the chromatogram. Sometimes the changes are easy to predict, and in other cases, the final result can be a surprise if all of the ramifications are not recognized and addressed. GC hardware requirements can also limit the type or size of column. Deviating from the recommended columns can result in poor chromatography.

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting*, P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

Dean Rood
Associate Editor