Gas Chromatography Problem Solving and Troubleshooting

Question:

I was using a split injector with a 1:100 split ratio for the GC analysis of 100-ng/µL drug samples. To obtain better detection limits, I changed to the splitless mode and kept the same temperature and carrier gas conditions. Using a 0.5-min purge on time and a 1-µL injection of a 10-ng/µL standard, only a large solvent front and a few extremely small peaks were obtained. I expected the peaks to be much larger than the ones obtained with the split injector. What is wrong with my splitless injector?

Answer:

After examining the column and injector parameters, there is probably nothing wrong with the splitless injector. The results were contrary to what is expected because splitless injections should deposit a much larger amount of the sample into the column—in theory approximately 10 times the amount in this case. There are other factors that can influence injector performance. In this case, the poor result was primarily caused by the incompatibility of very small diameter columns and splitless injectors.

For splitless injectors, the carrier gas flow rate in the injector at the time of injection is the same as the column carrier gas flow rate. Upon injection, the sample is vaporized, and it expands and fills the injector liner. The vaporized sample is then transferred from the liner and into the column by the flowing carrier gas. For columns with inner diameters of 0.25 mm or larger, carrier gas flow rates of 1 mL/min or higher are typical. Most of the liner volume is swept by the carrier gas, thus a significant amount of the vaporized sample is transferred into the column. If the carrier gas flow rate is very low, only a small fraction of the liner volume is swept, and a much smaller portion of the sample is transferred into the column. For split injectors, the carrier gas flow rate in the injector is the sum of the column and split vent flow rates. Total flow rates of 20 mL/min (and usually much higher) are typical. The liner volume is swept numerous times during the sample transfer process, and most of the sample is transferred into the column or out of the split vent. Sample transfer rates and swept liner volumes are much lower for splitless injectors than for split injectors.

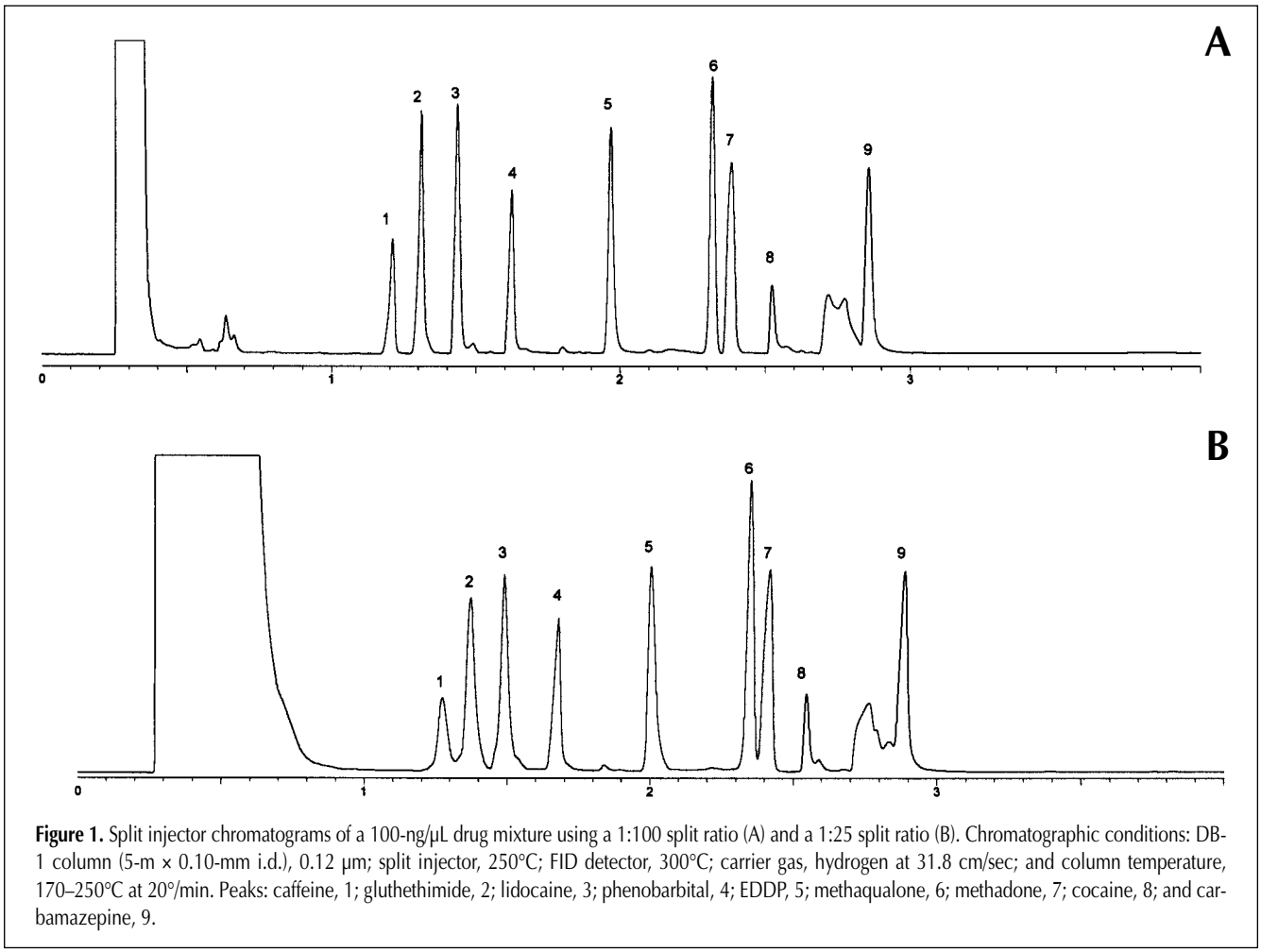

Figure 1. Split injector chromatograms of a 100-ng/µL drug mixture using a 1:100 split ratio (A) and a 1:25 split ratio (B). Chromatographic conditions: DB-1 column (5-m × 0.10-mm i.d.), 0.12 µm; split injector, 250°C; FID detector, 300°C; carrier gas, hydrogen at 31.8 cm/sec; and column temperature, 170–250°C at 20°/min. Peaks: caffeine, 1; gluthethimide, 2; lidocaine, 3; phenobarbital, 4; EDDP, 5; methaqualone, 6; methadone, 7; cocaine, 8; and carbamazepine, 9.

The hydrogen carrier gas flow rate in the injector for the 5-m × 0.10-mm-i.d. column being used was approximately 0.2 mL/min. For a 1:100 split ratio, the total flow was approximately 20 mL/min. Although this was at the low end of the recommended flow rate range, an acceptable chromatogram was obtained (Figure 1A). As the carrier gas flow rate in the injector decreased below 20 mL/min, noticable degradation in peak shapes and widths occurred. At a 1:25 split ratio, the total flow rate in the injector was approximately 5 mL/min. This split ratio resulted in broader peaks and a larger solvent front (Figure 1B). For the splitless injection, the injector was swept by approximately 0.2 mL/min of carrier gas. At a 0.5-min purge time, a total of 0.1 mL of carrier gas flowed through the injector before the purge function occurred (which effectively ended sample transfer into the column). Although the liner volume was near 0.8 mL, approximately $^1/_8$ of the liner was swept by the carrier gas and only a very small portion of the sample was transferred into the column. This was the primary reason for the lack of peak size in the splitless injection—only a very small fraction of the sample was transferred into the 0.10-mm-i.d. column because of the very low flow rate of the carrier gas in the injector.

Examining a splitless injection by the use of a larger diameter column further illustrated this concept. Using a 15-m × 0.25-mm i.d., the hydrogen carrier gas flow rate in the injector was approximately 0.9 mL/min. For a 0.5-min purge on time, 0.45 mL of the carrier gas flowed through the liner. This was sufficient volume to transfer enough of the sample into the column so that acceptable sized peaks could be obtained (Figure 2).

A lower initial oven temperature of 100°C was used to generate the splitless injector chromatogram in Figure 2 (compared to 170°C). One of the guidelines for splitless injections is to use an initial oven temperature that is at least 10°C below the boiling point of the sample solvent or at least 150°C below the boiling point of the sample analytes. Using an excessively high initial oven temperature may result in broad or distorted peak shapes. Broader peaks reduce detection limits because the peaks become shorter as they increase in width. Combined with the very low sample amount transferred into the column and the broad peaks from using an excessively high initial oven temperature, the peaks for the splitless injector chromatogram were much smaller (i.e., shorter) than for the 1:100 split injector chromatogram using a 0.10-mm-i.d. column. This problem was not as severe with larger diameter capillary columns.

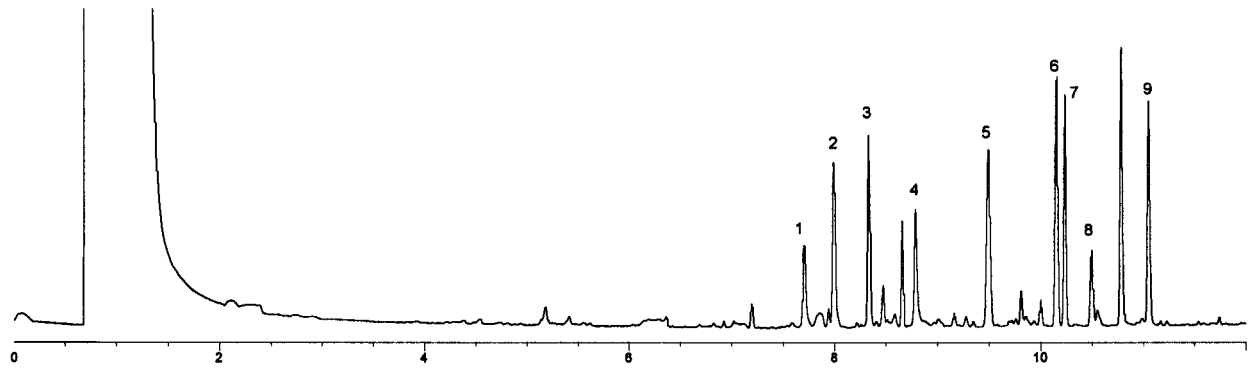

Figure 2. Splitless injector chromatogram of a 10-ng/µL drug mixture using a 0.25-mm-i.d. capillary column. Chromatographic conditions: DB-1 column (15-m × 0.25-mm i.d.), 0.25 µm; splitless injector, 250°C, 0.5-min purge on time; FID detector, 300°C; carrier gas, hydrogen at 38.9 cm/sec; column temperature, 100°C for 1 min, 100–265°C at 15°/min. Peaks: caffeine, 1; gluthethimide, 2; lidocaine, 3; phenobarbital, 4; EDDP, 5; methaqualone, 6; methadone, 7; cocaine, 8; and carbamazepine, 9.

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 or answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions or answers.

Dean Rood
Associate Editor