LC TROUBLESHOOTING

Column Heating and Resolution — A Case Study

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Careful control of column temperature may not be enough. How the column is heated can affect the method.

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The case study presented this month reminds us that it is often important to ask the question "how?" when trying to reproduce a method from another laboratory. The method may tell us to "mix A and B" or to "heat the column to 40 °C," but crucial information may be missing. We've all experienced times when the experimental outcome depends on how a task is performed. In the current example, the workers found that the specific technique used to heat the column had a significant influence on the results obtained by the method they were using. Although such situations can increase our understanding of the important variables in a method, they can often be avoided if the experimental setup and procedures are carefully described. When such detailed information is available, problems can be readily solved by comparing the problem system and reference instrument.

—JWD

COLLABORATION SPARKS INVESTIGATION

In a previous "LC Troubleshooting" column, we reported about the influence of the column-heater type and flow rate on baseline noise (1). In this installment of "LC Troubleshooting," we report on the influence of the column heater and flow rate on resolution. It is commonly known that column temperature can play an important role in chromatographic resolution. We found that the method of heating the column can also be an important influence on resolution, as can the mobile-phase flow rate, which affects heat transfer in the system. This investigation was undertaken after we organized a collaborative study on the liquid chromatographic (LC) separation of erythromycin using a published method and found that several of the laboratories involved reported difficulties in reaching the resolution limit.

The column (25 cm × 4.6 mm) was packed with 1000-Å porosity poly(styrene–divinylbenzene) particles (8-µm d_p) and had to be heated to 70 °C. The mobile phase was 3:16.5:5:75.5 acetonitrile–2-methyl-2-propanol–0.2 M phosphate buffer (pH 9.0)–water at a flow rate of 2.0 mL/min. UV detection was performed at 215 nm. A minimum resolution of 5.5 was required between erythromycin A and *N*-demethylerythromycin A to ensure sufficient separation of the major component from the impurities (2). Before the collaborative study was organized, the method had been used in five laboratories, and none reported difficulties (2).

INCONSISTENT RESULTS

When the collaborative study started, the flow rate was increased to 2.0 mL/min (it was 1.0 mL/min in the original method) because a reduction in the total analysis time was required. The organizing laboratory encountered no difficulties in using the higher flow rate. The laboratories reporting difficulties in obtaining the desired resolution were using a hot-air oven to heat the column to 70 °C. In our laboratory, column temperature was controlled by immersing the column in a water bath. The other laboratories' problems disappeared, however, when the flow rate was reduced to 1.0 mL/min.

HOT IS NOT ENOUGH

Because of the interlaboratory discrepancies, we investigated the influence of the heating device and flow rate on resolution. Three heating devices — a hot-air oven, a water jacket, and an immersion bath — were incorporated in one chromatographic apparatus. The hot-air oven was equipped with a laboratory-made metal heating block through which the tubing between the injector and the column passed before entering the oven. The preheating block functioned independently of the hot-air oven. Besides the preheating block, the oven could also be equipped with a coil of inlet tubing (0.5 m long) inside the oven to provide additional preheating of the mobile phase. The water jacket seals were fixed on the inlet and outlet column fittings and not on the tubing, thus leaving part of the endfitting in contact with ambient air.

We used these heating devices and flow rates of 1.0 mL/min and 2.0 mL/min to measure the resolution. The results in Table I show that the resolution improved most with the water bath, less with the water jacket, and least with the hot-air oven. The resolution ob-

TABLE I: Resolution Obtained Using Different Heating Devices Set at 70 °C

Flow Rate (mL/min)	Hot-Air Oven	Water Jacket	Water Bath
1.0	5.65	5.9	6.4
	5.8*		
	6.3†		
2.0	4.0	4.2	6.4
	5.0*		
	5.2†		

*With preheating block.
†With preheating block and a 50-cm coil inside the oven.

tained with the hot-air oven increased when the preheating device was used. Resolution also improved when a flow rate of 1.0 mL/min was used with the water jacket and hot-air oven; with the water bath, no change was observed when the 1.0-mL flow rate was used. These results led us to conclude that some heating devices are not suitable for adequately heating a column, certainly when high temperatures and high flow rates are used.

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In a subsequent experiment using the water bath at 65 °C instead of 70 °C and using a 2.0-mL/min flow rate, the resolution dropped from 6.4 to 5.7. This drop can be explained by reduced mass transfer between the stationary and mobile phases. This result also shows that the problem encountered when some heating devices are used probably is not simply the result of a reduction in temperature caused by poor heat exchange between the air and the column but rather is a result of the existence of a broad temperature gradient inside the column, which leads to peak broadening and consequent reduction of resolution.

This conclusion is supported by the fact that poor resolution was obtained when the water jacket was used even though the heat exchange between water and column must be comparable to that in the immersion bath. The apparent cause is that at the moment the mobile phase reaches the top of the column in the water jacket, it is not at the required temperature because the endfitting is inadequately heated. Inside the column, the mobile phase is in contact with the column wall and heats faster than the mobile phase in the center of the column, thus producing a temperature gradient. Using the hot-air oven to preheat the mobile phase, we were able to lessen the effect. The preheating applied, however, was insufficient to raise the resolution to a value comparable to that obtained when the water bath was used. Excessively increasing the length of the inlet tubing will cause peaks to broaden and thereby decrease resolution.

A SIMPLE SOLUTION

Using a simple immersion bath composed of a 35 cm × 25 cm × 15 cm bath with a plastic lid and an immersion heater, we observed no problems because the inlet tubing passed through the bath before entering the column, thus sufficiently preheating the mobile phase. In this configuration, the inlet fitting of the column was also heated adequately.

When this information was passed to a collaborative laboratory that had reported poor resolution when using a hot-air oven, the workers there were able to adequately preheat the mobile phase and reach the resolution limit (≥5.5). After making this correction, the same laboratory still reported a baseline noise problem. The existence of thermal noise problems caused by poor heat exchange before the mobile phase enters the detector has been discussed previously (1). The problem was solved by increasing the length of tubing between the column and the detector.

Thus, adequate heating of the column and subsequent cooling between the column and detector play an important role in LC methods that must be performed at increased temperatures.

REFERENCES

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