Liquid Chromatography Problem Solving and Troubleshooting

Question:

I am using a two-pump gradient system with a dynamic mixer to form my gradients. I used the procedure you presented in a 1994 article (1) to evaluate my system and obtained the result shown in Figure 1. It is my opinion that the system is not working properly. Do you agree, and if so, what is wrong?

Answer:

I concur with your opinion. But before I discuss the possible problem with your system, I would like to commend you on testing the system before using the gradient in an operational way. Obviously, if you had not tested your system, any results that you may have gathered would not have been transferable to another system, nor would they likely have been reproducible from run to run.

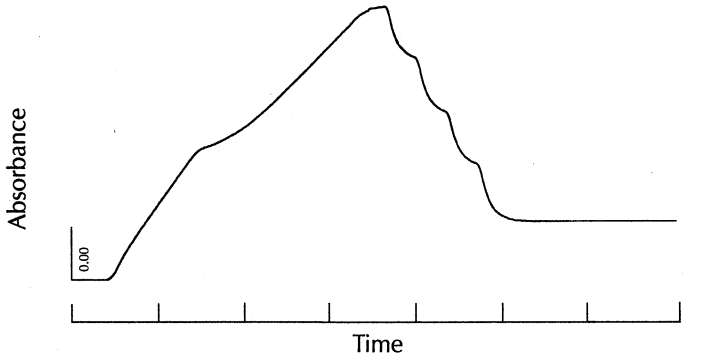

Figure 1. Gradient profile. Conditions: 100% A to 100% B in 45 min at 1.5 mL/min. At 100% B, return steps in 5 min using 20% increments. The A solvent is methanol, and the B solvent is 0.3% acetone in methanol.

The response curve you submitted for a linear-shaped gradient (Figure 1) appears to demonstrate that the gradient is not performing correctly. There are three possible sources of the problem: the pumps (one or both), the dynamic mixer, and the electronics. First determine whether each pump is operating correctly and whether there are any leaks in the system. Monitor the pressure trace during the gradient formation and observe if there is any indication of large pulsations, indicative of check valve malfunctioning. The pump accuracy testing can be done by measuring the flow rate pumping into a graduated cylinder for a fixed amount of time. From Figure 1, it looks as if the pump that delivered solvent B may not be delivering solvent correctly because there was a delay (perhaps exponential) of the response curve at approximately 50% A and B solvent.

If the pumps are working correctly, it is probable that the stirring bar inside the dynamic mixer may not be functioning correctly during the entire run, and, as a result, the mixer is actually behaving as a "dilution flask." This would account for the rounded shape during the up-slope region (near 50% A and B solvent) and the rounded shapes during the step return function. It should be noted to the readers that the final return is only to the 80% A, 20% B point, and that this is the reason the baseline does not go to the initial starting absorbance value. Open up the dynamic mixer and observe the condition of the stirring bar as well as its spinning performance. Replace the bar if necessary. If the pumps and the dynamic mixer are working correctly, the problem is in the electronics of the gradient controller. Assessment of the electronics is difficult to do without help, so call the manufacturer and follow their protocol.

Reference

1. B. Bidlingmeyer. Liquid chromatography problem solving and troubleshooting. *J. Chromatogr. Sci.* **32:** 164 (1994).

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.

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Associate Editor