Liquid Chromatography Problem Solving and Troubleshooting

Question

I am using a mobile phase of 50:50 acetonitrile and acetate buffer at pH 4.0 and am interested in separating two peaks on a C18 column. One of my compounds is water-soluble and the other is less so but soluble in methanol. On an endcapped C18, the first peak elutes at the void volume (1 min) and the second peak elutes at 6 min. However, using a nonendcapped C18 column, the first peak elutes at 3 min and the second peak elutes at 6 min. I have more that enough resolution between the peaks, so I am satisfied with the retention aspect; but, my problem is that the first peak has a tailing factor of 2.2 to 2.4 and the second peak has a tailing factor of 1.05. How do I get the tailing factor of the first peak to be 1.3 or better?

Answer

First off, are you sure that you have a problem here? If this is going to be a method for routine analysis and you have such great resolution, why are you worrying about the tailing factor? As long as you have adequate peak response to obtain good quantitation, there is no reason to concern yourself with the tailing factor. Remember that the tailing factor is only a figure of merit because when it is very large one peak might overlap with another peak or the peak response could be too low for adequate quantitation. You have neither of these two problems. So I would remind you of the old, wise saying that "better is the enemy of good enough."

My philosophy in method development is to structure the goals in a hierarchy with the top three being adequate retention, adequate resolution, and adequate quantitation. In addition to this general separation hierarchy, you seem to have answered the other questions such as, what degree of resolution do you require, how many compounds need to be resolved, and is a quantitative analysis required for each compound? If you cannot define the goals of the separation, then you will never know when you are done. So the biggest question is, are you done with the separation that you described?

Remember that developing a chromatographic method often involves making a succession of trade-offs that are necessary, because seldom can you settle on a perfect and ideal final procedure. In your case, you seem to require the underlying silanols to attain the retention of the first compound. It is never wise to develop a method in which the first peak is eluting at the void volume because everything that is not retained elutes there, and you can never guarantee that the peak is attributable solely to the sought for substance. Using the nonendcapped column, you have a method with the first peak eluting at a capacity factor of 2, and this is adequate for a rugged method. Furthermore, you have chosen a buffer at a pH of 4, so the silanols are not ionized; or if they are, they only have a very small ionic nature. So it appears that you have chosen your conditions appropriately. And because the resolution is so good, the tailing factor of 2.2 is not a red flag. The method seems to be good.

But should you wish to experiment on improving the peak shape, try adding a 0.1% triethylamine (TEA) to the mobile phase. The addition of a small amount of TEA will take time to condition the column and should effectively compete with the sample for the silalnols such that the peak shape should improve. However, it may compete too well, and the retention time will significantly drop. If this happens, the only choice is to make the mobile phase weaker in acetonitrile. I would not recommend doing all of this extra work because you seem to have accomplished a nice separation. However, this approach may offer you better peak shapes, but it will make the system a bit more complex.

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,* 6600 W. Touhy Ave., Niles, IL 60714-4516. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

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