Liquid Chromatography Problem Solving and Troubleshooting

Question

About six months ago, a very broad peak first started appearing in the chromatograms of our gradient elution reversed-phase methods. It is particularly troublesome in one of the methods. At the time, it was barely distinguishable from the baseline. However, with time it has continued to increase in size until it is now the largest peak in most of our chromatograms. In addition, after the first gradient run is complete, a second peak was observed. This peak was even less noticeable at first, but it is now also large. Since we have changed nothing in the assays, it is unclear what is causing the extra peaks. I have included two representative chromatograms that illustrate the problem. Do you have any suggestions?

Answer

Although background contamination can arise from several sources, the water–buffers that are used to prepare the eluent is generally the most common source. Traces of a single background contaminate or multiple contaminates in an aqueous buffer is less noticeable under isocratic operations. Because this problem manifests itself as a slightly higher than normal baseline signal, background contamination often goes unnoticed. This is especially true with modern detectors/data systems that auto zero at the beginning of each new injection/run. If the level of contamination is constant with time, it may never be noticed if one only runs isocratic separations.

In the case of gradient elution runs, background contamination is often readily apparent, appearing as drifting baselines and unidentified peaks in the chromatograms. Likewise, it is not that unusual to have the largest peak or peaks appear in the first run in a series of runs. This results from the fact that prior to beginning a new set of experiments, most individuals wash their columns with an organic rich eluent and then condition it with the starting eluent for a much longer time than they use between repetitive gradient runs. To understand this process/effect, it is helpful to go through each step in carrying out a gradient elution experiment after the organic rich wash has been completed.

During the initial column conditioning step, the eluent's strength is lowest and any contamination that is not eluted begins to concentrate on the head of the column. Once the gradient is started and the organic component in the eluent begins increasing, a point is reached where the concentrated background contaminates begin migrating. The resulting peak that shows up unexpectedly in the chromatogram can vary in size and width depending on its concentration and sorption behavior. Between gradient runs, it is not unusual to employ some type of reverse gradient, often at an increased rate to reestablish the initial eluent conditions. Because of this, the concentration step is different in terms of the amount of contaminate exposure the column sees and how it is longitudinally distributed on the column. When the next run is carried out, the sorbed contaminate will again elute from the column, but it will be smaller in size and its shape and retention can change. The chromatograms included with your question (i.e., Figures 1A and 1B) are classic examples of this type of behavior. All gradient runs after the second will produce similar chromatograms because the reversed

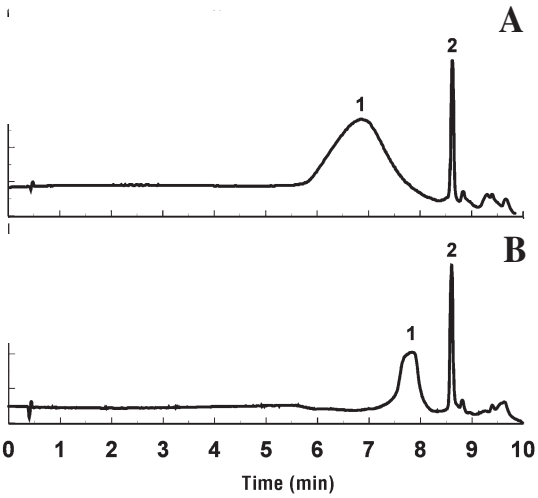

Figure 1. Gradient chromatograms showing the presence of an unknown contaminant (peak 1) eluting just before the compound of interest (peak 2). Chromatogram obtained from the first elution (A) and chromatogram from the second run (B). Subsequent chromatograms, which are not shown, should be similar to B.

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.

Roger K. Gilpin
Associate Editor

gradient profile and subsequent background contaminate buildup (i.e., its sorption profile) will be identical in size and distribution on the head of the column.

Although contamination can be introduced from several sources, the most likely cause of the peak(s) you are observing is a problem with the water you are using as the result of bacterial growth in your water purification system. A clear indication of this is that once the problem begins, it continues to increase at a faster and faster rate. Although you did not indicate how you are obtaining the water that is being used to make your chromatographic eluents, I assume that it is being produced in-house using one of the commercially available high purity ion-exchange units. In most cases, these units are very reliable and trouble free. However, depending on how often you use the unit, microorganisms can be deposited and grow on the resins. Once this starts, the rate of growth generally increases with time. This problem is greatest if the water purification system (i.e., ion-exchange resins) is used infrequently.

If you are experiencing problems with your water purification unit, you also should be seeing this problem show up in your aqueous buffers, especially those that are phosphate based. The problem of microorganisms growth in phosphate buffers is a well known problem, but with contaminated water it is an even greater problem. Buffers that cloud very quickly are another indication of contaminated water.

To solve your problem, you will need to clean (i.e., decontaminate) your water purification system and then change the ion-exchange and carbon cartridges. In terms of the cleaning procedures, you should consult the manufacturer of your equipment. They are well aware of the microorganism problem and are familiar with any hardware constraints in terms of their particular system and what chemical(s) you can safely use.