# Liquid Chromatography Problem Solving and Troubleshooting

***Question:***

For some of the HPLC analyses that I run, I observe rounded peaks. Some collegues say I should ignore this, and others feel that something is wrong in the chromatography, but they have no idea what it could be. How do I troubleshoot this problem?

***Answer:***

Chromatographic peaks should be sharp; rounded peaks mean something is amiss. The cause of rounded peaks can be one of several, and each possible item should be evaluated as a potential cause. This situation is more complex than many troubleshooting activities because the cause could be a hardware problem and/or a chromatographic problem. Therefore, evaluate the hardware first; only when the hardware is known to be working properly can the chromatographic influences be accurately observed.

Initially, consider the detector. Change the time constant of the detector and rerun the analysis. If the time constant is set too high, this can cause rounded peaks. The time constant should be as low as possible to obtain a lively signal with chatter on the baseline (as opposed to high noise). Also, remember that some data systems may have smoothing algorithms in the software and essentially act as "time constants" with respect to the chromatogram. If your software has such smoothing routines, these should also be tested for their effect on possible rounding of the peaks. If the electronic gain of the detector's output amplifier is too low, this could also cause rounded peaks. Therefore, check the gain of the detector. Of course, if you are using a recorder, the above comments about time constants and gain are revenant to the recorder, and these variables should be evaluated for their effect on peak shapes.

The next step should be to evaluate whether the detector is operating outside of its linear range. For example, if you are using a UV detector and the output signal is greater than 2 absorbance units, it is likely that the detector is operating in its nonlinear range. Each detector has a linear range, and it is important to be in this range. A previous Troubleshooting article dealt with determining this range (1). If this is the case, injecting a smaller amount of sample should result in sharper peaks. Reducing the sample size by a factor of 10 and observing the peak shape is a quick way of determining whether you are operating in the linear range of the detector.

Another possible cause of the rounded peak shape could be that the sample is overloading the column. Remember that it is possible to operate in the nonlinear range of the detector and not overload the column. Conversely, it is possible to overload the column and still be in the linear range of the detector. In order to test for column overload, inject the same sample volume using a 10-fold decrease in concentration and then, if possible, inject another sample using an additional 10-fold decrease in concentration. Observe the peak shapes. It is possible to have an overload due to the injected volume. This situation can be evaluated by injecting less volume. Inject a 5-µL and a 1-µL volume and oberve how the peak shapes compare with those obtained using your present sample size.

Another possible cause of the rounded peaks could be the use of an inappropriate solvent in the sample injection. Occasionally, samples can be injected in solvents other than the mobile phase; however, this approach can result in deleterious performance. To evaluate this variable, dissolve the sample in the mobile phase; then inject and observe the peak shapes. Of course, your situation could be one or a combination of the items listed above.

***Reference***

1. B.A. Bidlingmeyer. Liquid chromatography problem solving and troubleshooting. *J. Chromatogr. Sci.* **31:** 294 (1993).

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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